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(54) Title: CDR-GRAFTED ANTI-TISSUE FACTOR ANTIBODIES AND METHODS OF USE THEREOF

(57) Abstract

The present invention provides CDR-grafted antibodies against human tissue factor that retain the high binding affinity of rodent monoclonal antibodies against tissue factor but have reduced immunogenicity. The present humanized antibodies are potent anticoagulants and are thus useful in the treatment and prophylaxis of human thrombotic disease. The invention also provides methods of making the CDR-grafted antibodies and pharmaceutical compositions for the attenuation or prevention of coagulation.

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CDR-GRAFTED ANTI-TISSUE FACTOR

1 ANTIBODIES AND METHODS OF USE THEREOFFIELD OF THE INVENTION

5 Monoclonal antibodies capable of inhibiting tissue factor (TF) are useful as anticoagulants. Conventional rodent monoclonal antibodies, however, have limited use in human therapeutic and diagnostic applications due to immunogenicity and short serum half-life. The present invention provides CDR-grafted monoclonal antibodies against TF that retain the high binding affinity of rodent antibodies but have reduced immunogenicity. The present humanized antibodies are potent anticoagulants and are thus useful in the 10 treatment and prophylaxis of human thrombotic disease. The invention also provides methods of making the CDR-grafted antibodies and pharmaceutical compositions for the attenuation or prevention of coagulation.

15

20 BACKGROUND OF THE INVENTION

The coagulation of blood involves a cascading series of reactions leading to the formation of fibrin. The coagulation cascade consists of two overlapping pathways, both of which are required for hemostasis. 25 The intrinsic pathway comprises protein factors present in circulating blood, while the extrinsic pathway requires tissue factor, which is expressed on the cell surface of a variety of tissues in response to vascular injury. Davie et al., 1991, Biochemistry 30:10363. 30 Agents that interfere with the coagulation cascade, such

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as heparin and coumarin derivatives, have well-known
1 therapeutic uses in the prophylaxis of venous
thrombosis. Goodman and Gilman, eds., 1980, The
Pharmacological Basis of Therapeutics, MacMillan
Publishing Co., Inc., New York.

5 Tissue factor (TF) has been investigated as a
target for anticoagulant therapy. TF is a membrane
glycoprotein that functions as a receptor for factor VII
and VIIa and thereby initiates the extrinsic pathway of
the coagulation cascade in response to vascular injury.
10 In addition to its role in the maintenance of hemostasis
by initiation of blood clotting, TF has been implicated
in pathogenic conditions. Specifically, the synthesis
and cell surface expression of TF has been implicated in
vascular disease (Wilcox et al., 1989, Proc. Natl. Acad.
15 Sci. 86:2839) and gram-negative septic shock (Warr et
al., 1990, Blood 75:1481).

Ruf et al. (1991, Thrombosis and Haemostasis
66:529) characterized the anticoagulant potential of
murine monoclonal antibodies against human TF. The
20 inhibition of TF function by most of the monoclonal
antibodies that were assessed was dependent upon the
dissociation of the TF/VIIa complex that is rapidly
formed when TF contacts plasma. Such antibodies were
thus relatively slow inhibitors of TF in plasma. One
25 monoclonal antibody, TF8-5G9, was capable of inhibiting
the TF/VIIa complex without dissociation of the complex,
thus providing an immediate anticoagulant effect in
plasma. Ruf et al. suggest that mechanisms that
inactivate the TF/VIIa complex, rather than prevent its
30 formation, may provide strategies for interruption of
coagulation in vivo.

The therapeutic use of monoclonal antibodies

1 against TF is limited in that currently available monoclonals are of rodent origin. The use of rodent antibodies in human therapy presents numerous problems, the most significant of which is immunogenicity.

5 Repeated doses of rodent monoclonal antibodies have been found to elicit an anti-immunoglobulin response termed human anti-mouse antibody (HAMA), which can result in immune complex disease and/or neutralization of the therapeutic antibody. See, e.g., Jaffers et al. (1986)

10 Transplantation 41:572. While the use of human monoclonal antibodies would address this limitation, it has proven difficult to generate large amounts of human monoclonal antibodies by conventional hybridoma technology.

15 Recombinant technology has been used in an effort to construct "humanized" antibodies that maintain the high binding affinity of rodent monoclonal antibodies but exhibit reduced immunogenicity in humans. Chimeric antibodies have been produced in which the

20 variable (V) region of a mouse antibody is combined with the constant (C) region of a human antibody in an effort to maintain the specificity and affinity of the rodent antibody but reduce the amount of protein that is non-human and thus immunogenic. While the immune response

25 to chimeric antibodies is generally reduced relative to the corresponding rodent antibody, the immune response cannot be completely eliminated, because the mouse V region is capable of eliciting an immune response.

Lobuglio et al. (1989) Proc. Natl. Acad. Sci. 86:4220;

30 Jaffers et al. (1986) Transplantation 41:572.

In a recent approach to reducing immunogenicity of rodent antibodies, only the rodent complementarity determining regions (CDRs), rather than the entire V domain, are transplanted to a human antibody. Such humanized antibodies are known as CDR-grafted antibodies. CDRs are regions of hypervariability in the V regions that are flanked by relatively conserved regions known as framework (FR) regions. Each V domain contains three CDRs flanked by four FRs. The CDRs fold to form the antigen binding site of the antibody, while the FRs support the structural conformations of the V domains. Thus by transplanting the rodent CDRs to a human antibody, the antigen binding domain can theoretically also be transferred. Owens *et al.* (1994) J. Immunol. Methods 168:149 and Winter *et al.* (1993) Immunology Today 14:243 review the development of CDR-grafted antibodies.

Orlandi *et al.* (1989) Proc. Natl. Acad. Sci. USA 86:3833 constructed a humanized antibody against the relatively simple hapten nitrophenacetyl (NP). The CDR-grafted antibody contained mouse CDRs and human FRs, and exhibited NP binding activity similar to the native mouse antibody. However, the construction of CDR-grafted antibodies recognizing more complex antigens has resulted in antibodies having binding activity significantly lower than the native rodent antibodies. In numerous cases it has been demonstrated that the mere introduction of rodent CDRs into a human antibody background is insufficient to maintain full binding activity, perhaps due to distortion of the CDR conformation by the human FR.

For example, Gorman et al. (1991) Proc. Natl.

- 1 Acad. Sci. 88:4181 compared two humanized antibodies against human CD4 and observed considerably different avidies depending upon the particular human framework region of the humanized antibody. Co et al. (1991)
- 5 Proc. Natl. Acad. Sci. USA 88:2869 required a refined computer model of the murine antibody of interest in order to identify critical amino acids to be considered in the design of a humanized antibody. Kettleborough et al. (1991) Protein Engineering 4:773 report the
- 10 influence of particular FR residues of a CDR-grafted antibody on antigen binding, and propose that the residues may directly interact with antigen, or may alter the conformation of the CDR loops. Similarly, Singer et al. (1993) J. Immunol. 150:2844 report that
- 15 optimal humanization of an anti-CD18 murine monoclonal antibody is dependent upon the ability of the selected FR to support the CDR in the appropriate antigen binding conformation. Accordingly, recreation of the antigen-binding site requires consideration of the potential
- 20 intrachain interactions between the FR and CDR, and manipulation of amino acid residues of the FR that maintain contacts with the loops formed by the CDRs. While general theoretical guidelines have been proposed for the design of humanized antibodies (see, e.g., Owens
- 25 et al.), in all cases the procedure must be tailored and optimized for the particular rodent antibody of interest.

There is a need in the art for humanized antibodies with reduced immunogenicity and comparable

- 30 binding affinity relative to the parent rodent antibody for various therapeutic applications. In particular,

there is a need for a humanized antibody against human
1 tissue factor having anticoagulant activity and useful
in the treatment and prevention of thrombotic disease.

SUMMARY OF THE INVENTION

5

The present invention is directed to CDR-grafted antibodies capable of inhibiting human tissue factor wherein the CDRs are derived from a non-human monoclonal antibody against tissue factor and the FR and 10 constant (C) regions are derived from one or more human antibodies. In a preferred embodiment, the murine monoclonal antibody is TF8-5G9.

In another embodiment, the present invention provides a method of producing a CDR-grafted antibody 15 capable of inhibiting human tissue factor which method comprises constructing one or more expression vectors containing nucleic acids encoding CDR-grafted antibody heavy and light chains, transfecting suitable host cells with the expression vector or vectors, culturing the 20 transfected host cells, and recovering the CDR-grafted antibody.

The present invention also provides a method of attenuation of coagulation comprising administering a CDR-grafted antibody capable of inhibiting human tissue 25 factor to a patient in need of such attenuation.

The present invention further provides a method of treatment or prevention of thrombotic disease comprising administering a CDR-grafted antibody capable of inhibiting human tissue factor to a patient in need 30 of such treatment or prevention. In a preferred

embodiment, the thrombotic disease is intravascular
1 coagulation, arterial restenosis or arteriosclerosis.

Another embodiment of the present invention is
directed to a pharmaceutical composition comprising CDR-
grafted antibodies capable of inhibiting human tissue
5 factor and further comprising a pharmaceutically
acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

10 Fig. 1 provides the nucleotide and deduced
amino acid sequences of the heavy chain of murine
monoclonal antibody TF8-5G9.

Fig. 2 provides the nucleotide and deduced
amino acid sequences of the light chain of murine
15 monoclonal antibody TF8-5G9.

Fig. 3 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR1 x TF8LCDR1 to bind to
human tissue factor and to compete with murine
monoclonal antibody TF85G9 for binding to tissue factor.
20 Solid symbols indicate direct binding of TF8HCDR1 x
TF8LCDR1 and the positive control chimeric TF85G9 to
tissue factor. Open symbols indicate competition
binding of TF8HCDR1 x TF8LCDR1 or chimeric TF85G9 with
murine monoclonal antibody TF85G9.

25 Fig. 4 presents the DNA sequence of expression
vector pEe6TF8HCDR20 and the amino acid sequence of the
coding regions of the CDR-grafted heavy chain TF8HCDR20.

Fig. 5 presents the DNA sequence of expression
vector pEe12TF8LCDR3 and the amino acid sequence of the
30 coding regions of the CDR-grafted light chain TF8LCDR3.

Fig. 6 is a graph depicting the ability of
1 CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to bind to
human tissue factor.

Fig. 7 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to compete
5 with murine monoclonal antibody TF85G9 for binding to
tissue factor.

Fig. 8 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to inhibit
factor X activation.

10 Fig. 9 provides expression vector
pEe6TF8HCDR20 resulting from the subcloning of CDR-
grafted heavy chain TF8HCDR20 into myeloma expression
vector pEehCMV-BglI. The following abbreviations are
used: VH is the CDR-grafted heavy chain variable
15 region; Cy4 is the human IgG4 constant region; pA is the
polyadenylation signal; ampR is the β -lactamase gene;
and hCMV is human cytomegalovirus.

Fig. 10 provides expression vector
pEe12TF8LCDR3 resulting from the subcloning of CDR-
20 grafted light chain TF8LCDR3 into myeloma expression
vector pEe12. The following abbreviations are used: VL
is the CDR-grafted light chain variable region; CK is
the human kappa constant region; SVE is the SV40 early
promoter; GS is glutamine synthetase cDNA. Other
25 abbreviations are as noted in Fig. 9.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides CDR-grafted
30 antibodies capable of inhibiting human tissue factor
wherein the CDRs are derived from a non-human monoclonal

antibody against tissue factor and the FR and C regions
1 are derived from one or more human antibodies. The
present invention further provides methods of making and
using the subject CDR-grafted antibodies.

In accordance with the present invention, the
5 CDR-grafted antibody is an antibody in which the CDRs
are derived from a non-human antibody capable of binding
to and inhibiting the function of human tissue factor,
and the FR and C regions of the antibody are derived
from one or more human antibodies. The CDRs derived
10 from the non-human antibody preferably have from about
90% to about 100% identity with the CDRs of the non-
human antibody, although any and all modifications,
including substitutions, insertions and deletions, are
contemplated so long as the CDR-grafted antibody
15 maintains the ability to bind to and inhibit tissue
factor. The regions of the CDR-grafted antibodies that
are derived from human antibodies need not have 100%
identity with the human antibodies. In a preferred
embodiment, as many of the human amino acid residues as
20 possible are retained in order than immunogenicity is
negligible, but the human residues, in particular
residues of the FR region, are substituted as required
and as taught hereinbelow in accordance with the present
invention. Such modifications as disclosed herein are
25 necessary to support the antigen binding site formed by
the CDRs while simultaneously maximizing the
humanization of the antibody.

Non-human monoclonal antibodies against human
tissue factor from which the CDRs can be derived are
30 known in the art (Ruf *et al.*, 1991; Morrisey *et al.*,
1988, Thrombosis Research 52:247) or can be produced by

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well-known methods of monoclonal antibody production
1 (see, e.g. Harlow et al., eds., 1988, Antibodies, A
Laboratory Manual, Cold Spring Harbor Laboratories, Cold
Spring Harbor, New York). Purified human tissue factor
against which monoclonal antibodies can be raised is
5 similarly well-known (Morrisey et al., 1987, Cell
50:129) and available to the skilled artisan. Murine
monoclonal antibodies, and in particular murine
monoclonal antibody TF8-5G9 disclosed by Ruf et al. and
Morrisey et al., 1988, Thrombosis Research 52:247, and
10 U.S. Patent No. 5,223,427 are particularly preferred.

The ordinarily skilled artisan can determine
the sequences of the CDRs by reference to published
scientific literature or sequence databanks, or by
cloning and sequencing the heavy and light chains of the
15 antibodies by conventional methodology. In accordance
with the present invention, the cDNA and amino acid
sequences of the heavy chain (SEQ ID NOS:1 and 2,
respectively) and light chain (SEQ ID NOS:3 and 4,
respectively) of murine monoclonal antibody TF8-5G9 are
20 provided. The cDNA and deduced amino acid sequence of
the murine TF8-5G9 heavy chain is provided at Figure 1.
The cDNA and deduced amino acid sequence of the murine
TF8-5G9 light chain is provided at Figure 2.

Each of the heavy and light chain variable
25 regions contain three CDRs that combine to form the
antigen binding site. The three CDRs are surrounded by
four FR regions that primarily function to support the
CDRs. The sequences of the CDRs within the sequences of
the variable regions of the heavy and light chains can
30 be identified by computer-assisted alignment according
to Kabat et al. (1987) in Sequences of Proteins of

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Immunological Interest, 4th ed., United States

1 Department of Health and Human Services, US Government
Printing Office, Washington, D.C., or by molecular
modeling of the variable regions, for example utilizing
the ENCAD program as described by Levitt (1983) J. Mol.
5 Biol. 168:595.

In a preferred embodiment the CDRs are derived
from murine monoclonal antibody TF8-5G9. The preferred
heavy chain CDRs have the following sequences:

10 CDR1 DDYMH (SEQ ID NO:5)
CDR2 LIDPENGNTIYDPKFQG (SEQ ID NO:6)
CDR3 DNSYYFDY (SEQ ID NO:7)

15 The preferred light chain CDRs have the following
sequences:

20 CDR1 KASQDIRKYLN (SEQ ID NO:8)
CDR2 YATSLAD (SEQ ID NO:9)
CDR3 LQHGESPYT (SEQ ID NO:10)

25 The sequences of the CDRs of the murine or other non-
human antibody, and in particular the sequences of the
CDRs of TF8-5G9, may be modified by insertions,
substitutions and deletions to the extent that the CDR-
25 grafted antibody maintains the ability to bind to and
inhibit human tissue factor. The ordinarily skilled
artisan can ascertain the maintenance of this activity
by performing the functional assays described
hereinbelow. The CDRs can have, for example, from about
30 50% to about 100% homology to the CDRs of SEQ ID NOS:5-
10. In a preferred embodiment the CDRs have from about

80% to about 100% homology to the CDRs of SEQ ID NOS:5-1 10. In a more preferred embodiment the CDRs have from about 90% to about 100% homology to the CDRs of SEQ ID NOS:5-10. In a most preferred embodiment the CDRs have from about 100% homology to the CDRs of SEQ ID NOS:5-10.

5 The FR and C regions of the CDR-grafted antibodies of the present invention are derived from one or more human antibodies. Human antibodies of the same class and type as the antibody from which the CDRs are derived are preferred. The FR of the variable region of 10 the heavy chain is preferably derived from the human antibody KOL (Schmidt et al., 1983, Hoppe-Seyler's Z. Physiol. Chem. 364:713) The FR of the variable region of the light chain is preferably derived from the human antibody REI (Epp et al., 1974, Eur. J. Biochem. 45:513). In accordance with the present invention, it has been discovered that certain residues of the human FR are preferably replaced by the corresponding residue of the non-human antibody from which the CDRs are derived. For example, certain FR residues of TF8-5G9 20 are preferably retained to achieve optimal binding to antigen.

For convenience, the numbering scheme of Kabat et al. has been adopted herein. Residues are designated by lower case numbers or hyphens as necessary to conform 25 the present sequences to the standard Kabat numbered sequence.

In accordance with the present invention, residues that are retained in the FR region, i.e residues that are not replaced by human FR residues, are 30 determined according to the following guidelines. Residues that are idiosyncratic to the parent antibody,

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e.g. TF8-5G9, relative to a human consensus sequence of 1 Kabat *et al*, are retained. Residues of the parent antibody that are in agreement with the consensus sequence are retained if the corresponding residue of the human antibody, e.g. KOL or REI, is idiosyncratic. 5 Residues that are part of the antibody loop canonical structures defined by Chothia *et al*. (1989) Nature 342:877, such as residue 71 of the heavy and light chains, are retained. FR residues predicted to form loops, such as residues 28-30 of the heavy chain, are 10 retained. FR residues predicted to influence the conformation of the CDRs such as residues 48 and 49 preceding CDR2 of the heavy chain, are retained. Residues that have been demonstrated to be critical in the humanization of other antibodies may also be 15 retained. The foregoing guidelines are followed to the extent necessary to support the antigen binding site formed by the CDRs while simultaneously maximizing the humanization of the antibody.

The amino acid sequence of a representative 20 CDR-grafted heavy chain variable region derived from murine monoclonal antibody TF8-5G9 and human antibody KOL is shown below. The CDR-grafted heavy chain is designated TF8HCDR1; murine residues were retained in the FR at residues 6, 17, 23, 24, 28, 29, 30, 48, 49, 25 68, 71, 73, 78, 88 and 91. CDRs are underlined.

	10	20	30	35ab	50
QVQLVQSGGG	VVQPGRLRL	SCKASGFNIK	<u>DYYMH</u> --WVR	QAPGKGLEWIG	
52abc	60	70	80	82abc	90
LIDP--ENGNTIYD	<u>PKFQGRFSIS</u>	ADTSK--NTAFL	QMDSLRPEDTAVY		
100	110				
30	YCARDNSYYF	<u>DYWGQGTPVT</u>	VSS	(SEQ ID NO:11)	

The amino acid sequence of a representative
1 CDR-grafted light chain variable region derived from
murine monoclonal antibody TF8-5G9 and human antibody
REI is shown below. The CDR-grafted light chain is
designated TF8LCDR1; murine residues were retained in
5 the FR at residues 39, 41, 46 and 105. CDRs are
underlined.

	10	20	30	40	50
	DIQMTQSPSS	LSASVGDRVT	<u>ITCKASQDIR</u>	KYLNWYQQK	WKAPKTLIYY
10	60	70	80	90	100
	<u>ATSLADGVPS</u>	RFSGSGSGTD	YTFTISSLQP	<u>EDIATYYCLO</u>	<u>HGESPYTFGQ</u>
	GTKLEITR (SEQ ID NO:12)				

A CDR-grafted antibody containing variable
15 regions TF8HCDR1 and TF8LCDR1 has been demonstrated in
accordance with the present invention to be as effective
as murine monoclonal antibody TF8-5G9 in binding to
human tissue factor. It has been further discovered in
accordance with the present invention, by examination of
20 the molecular structure of murine monoclonal antibody
TF8-5G9, and by design, construction, and analysis of
CDR-grafted antibodies, that the FR regions can be
further humanized without the loss of antigen binding
activity. In particular, the FR region may retain the
25 human FR residue at residues 6, 17, 68, 73 and 78 of the
heavy chain, and residues 39, 41, 46 and 105 of the
light chain, with maintenance of antigen binding
activity.

In a most preferred embodiment, the heavy
30 chain variable region contains a FR derived from human
antibody KOL in which murine monoclonal antibody TF8-5G9

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residues are retained at amino acids 23, 24, 28, 29, 30,
1 48, 49, 71, 88 and 91. The preferred heavy chain
variable region is designated TF8HCDR20 and has the
following sequence.

5 10 20 30 35ab 50
QVOLVESGGG VVQPGRLRL SCKASGFNIK DYYMH--WVR QAPGKGLEWIGL

52abc 60 70 80 82abc 90 100
IDP--ENGNTIYD PKFQGRFTIS ADNSKNTLFL QMDSLRPEDTAVY YCARDNSYYF

10 110
DYWGQGTPVT VSS (SEQ ID NO:13)

15 In a most preferred embodiment, the light
chain variable region contains a FR derived from human
antibody REI in which murine monoclonal antibody TF8-5G9
residues are retained at amino acids 39 and 105. The
preferred light chain variable region is designated
TF8LCDR20 and has the following sequence.

20 10 20 30 40 50
DIQMTQSPSS LSASVGDRVT ITCKASQDIR KYLNWYQQKP GKAPKLLIYY
60 70 80 90 100
ATSLADGVPS RFSGSGSGTD YTFTISSLQP EDIATYYCLO HGESPYTFGQ
GTKLEITR (SEQ ID NO:14)

25 It is within the ken of the ordinarily skilled
artisan to make minor modifications of the foregoing
sequences, including amino acid substitutions, deletions
and insertions. Any such modifications are within the
scope of the present invention so long as the resulting
CDR-grafted antibody maintains the ability to bind to
30 and inhibit human tissue factor. The ordinarily skilled
artisan can assess the activity of the CDR-grafted

antibody with reference to the functional assays
1 described hereinbelow.

The human constant region of the CDR-grafted antibodies of the present invention is selected to minimize effector function. The intended use of the 5 CDR-grafted antibodies of the present invention is to block the coagulation cascade by inhibition of tissue factor, and thus antibody effector functions such as fixation of complement are not desirable. Antibodies with minimal effector functions include IgG2, IgG4, IgA, 10 IgD and IgE. In a preferred embodiment of the present invention, the heavy chain constant region is the human IgG4 constant region, and the light chain constant region is the human IgG4 kappa constant region.

In that effector functions may not be 15 desirable for therapeutic uses, the present invention further contemplates active fragments of the CDR-grafted antibodies, and in particular Fab fragments and $F(ab')_2$ fragments. Active fragments are those fragments capable of inhibiting human tissue factor. Fab fragments and 20 $F(ab')_2$ fragments may be obtained by conventional means, for example by cleavage of the CDR-grafted antibodies of the invention with an appropriate proteolytic enzyme such as papain or pepsin, or by recombinant production. The active fragments maintain the antigen binding sites 25 of the CDR-grafted antibodies and thus are similarly useful therapeutically.

The ability of the CDR-grafted antibodies designed and constructed as taught in accordance with the present invention to bind and inhibit human tissue 30 factor can be assessed by functional assays. For example, in a rapid and convenient assay, expression

vectors containing nucleic acids encoding the CDR-
1 grafted heavy and light chains can be co-transfected
into suitable host cells and transiently expressed. The
resulting antibodies can be assessed by standard assays
for ability to bind human tissue factor, and for ability
5 to compete for binding to tissue factor with the non-
human antibody from which the CDRs are derived.

For example, transient expression of nucleic
acids encoding the CDR-grafted heavy and light chains in
COS cells provides a rapid and convenient system to test
10 antibody gene expression and function. Nucleic acids
encoding the CDR-grafted heavy and light chains,
respectively, are cloned into a mammalian cell
expression vector, for example pSG5, described by Green
et al. (1988) Nucleic Acids Res. 16:369 and commercially
15 available from Stratagene Cloning Systems, La Jolla, CA.
The pSG5 expression vector provides unique restriction
sites for the insertion of the heavy and light chain
genes, and in vivo expression is under the control of
the SV40 early promoter. Transcriptional termination is
20 signaled by the SV40 polyadenylation signal sequence.

The pSG5-based expression vectors containing
nucleic acids encoding the heavy and light chains are
cotransfected into COS cells and cultured under
conditions suitable for transient expression. Cell
25 culture media is then harvested and examined for
antibody expression, for example by an enzyme linked
immunosorbent assay (ELISA), to determine that suitable
levels of antibody have been produced. An ELISA may
then be used to assess the ability of the CDR-grafted
30 antibody to bind to human tissue factor. Human tissue
factor is immobilized on a microtiter plate and the COS

cell supernatant containing the CDR-grafted antibody is
1 added followed by an incubation at room temperature for
about one hour. The plates are then washed with a
suitable detergent-containing buffer such as phosphate
buffered saline (PBS)/Tween, followed by the addition of
5 the components of a suitable detection system. For
example, horseradish peroxidase conjugated goat anti-
human kappa chain polyclonal antibody is added, followed
by washing, followed by addition of substrate for
horseradish peroxidase, and detection. The CDR-grafted
10 antibodies within the scope of the present invention are
those which are capable of binding to human tissue
factor to a degree comparable to the non-human antibody
from which the CDRs are derived as determined by the
foregoing assay.

15 The ability of the CDR-grafted antibodies to
inhibit the activity of human tissue factor in vivo can
be conveniently assessed by the following in vitro assay
that mimics in vivo coagulation events. In response to
vascular injury in vivo, tissue factor binds to factor
20 VII and facilitates the conversion of factor VII to a
serine protease (factor VIIa). The factor VIIa-tissue
factor complex converts factor X to a serine protease
(factor Xa). Factor Xa forms a complex with factor Va
(from the intrinsic coagulation pathway), resulting in
25 the conversion of prothrombin to thrombin, which in turn
results in the conversion of fibrinogen to fibrin. In a
convenient in vitro functional assay, tissue factor is
incubated in the presence of factor VIIa and the CDR-
grafted anti-tissue factor antibody produced in the
30 transient expression system described above. Factor X
is added and the reaction mixture is incubated, followed

by an assay for factor Xa activity utilizing a
1 chromogenic substrate for factor Xa (Spectrozyme FXa,
American Diagnostica, Inc., Greenwich, CT). The ability
of the CDR-grafted antibody to inhibit factor X
activation thus provides a measure of the ability of the
5 CDR-grafted antibody to inhibit the activity of human
tissue factor.

The CDR-grafted antibodies within the scope of
the present invention are those which are capable of
inhibiting human tissue factor to a degree comparable to
10 the non-human antibody from which the CDRs are derived
as determined by the foregoing assay. In one
embodiment, the CDR-grafted antibody has at least 50% of
the inhibitory activity of TF8-5G9 for human tissue
factor. In a preferred embodiment, the CDR-grafted
15 antibody has at least 70% of the inhibitory activity of
TF8-5G9 for human tissue factor. In a more preferred
embodiment, the CDR-grafted antibody has at least 80% of
the inhibitory activity of TF8-5G9 for human tissue
factor. In a most preferred embodiment, the CDR-grafted
20 antibody has at least 90% of the inhibitory activity of
TF8-5G9 for human tissue factor.

In another embodiment, the present invention
provides a method of producing a CDR-grafted antibody
capable of inhibiting human tissue factor. The method
25 comprises constructing an expression vector containing a
nucleic acid encoding the CDR-grafted antibody heavy
chain and an expression vector containing a nucleic acid
encoding the CDR-grafted antibody light chain,
transfected suitable host cells with the expression
30 vectors, culturing the transfected host cells under
conditions suitable for the expression of the heavy and

light chains, and recovering the CDR-grafted antibody.

1 Alternately, one expression vector containing nucleic acids encoding the heavy and light chains may be utilized.

Standard molecular biological techniques, for example as disclosed by Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual Cold Spring Harbor Press, Cold Spring Harbor, NY may be used to obtain nucleic acids encoding the heavy and light chains of the CDR-grafted antibodies of the present invention.

5 10 15 20 25 30 A nucleic acid encoding the CDR-grafted variable domain may be constructed by isolating cDNA encoding the antibody to be humanized, e.g. murine monoclonal antibody TF8-5G9, by conventional cloning methodology from the hybridoma producing the antibody, or by polymerase chain reaction (PCR) amplification of the variable region genes, as described for example by Winter et al., followed by site-directed mutagenesis to substitute nucleotides encoding the desired human residues into the FR regions. Alternately, the cDNA encoding the human antibody can be isolated, followed by site-directed mutagenesis to substitute nucleotides encoding the desired murine residues into the CDRs.

Nucleic acids encoding the CDR-grafted variable domain may also be synthesized by assembling synthetic oligonucleotides, for example utilizing DNA polymerase and DNA ligase. The resulting synthetic variable regions may then be amplified by PCR. Nucleic acids encoding CDR-grafted variable domains may also be constructed by PCR strand overlap methods that are known in the art and reviewed by Owens et al.

Accordingly, having determined the desired 1 amino acid sequences of the CDR-grafted variable domains in accordance with the present invention, the ordinarily skilled artisan can obtain nucleic acids encoding the variable domains. Further, the skilled artisan is aware 5 that due to the degeneracy of the genetic code, various nucleic acid sequences can be constructed that encode the CDR-grafted variable domains. All such nucleic acid sequence are contemplated by the present invention.

The nucleic acids encoding the CDR-grafted 10 variable domains are linked to appropriate nucleic acids encoding the human antibody heavy or light chain constant region. Nucleic acid sequences encoding human heavy and light chain constant regions are known in the art. It is within the ken of the ordinarily skilled 15 artisan to include sequences that facilitate transcription, translation and secretion, for example start codons, leader sequences, the Kozak consensus sequence (Kozak, 1987, J. Mol. Biol. 196:947) and the like, as well as restriction endonuclease sites to 20 facilitate cloning into expression vectors.

The present invention thus further provides nucleic acids encoding the heavy and light chains of CDR-grafted antibodies capable of inhibiting human tissue factor wherein the CDRs are derived from a murine 25 monoclonal antibody against tissue factor and the FR and C regions are derived from one or more human antibodies.

In accordance with the present invention, representative nucleic acids encoding CDR-grafted heavy and light chains were constructed. The CDR-grafted 30 heavy chain comprises a variable region containing FR regions derived from human antibody KOL and CDRs derived

from murine monoclonal antibody TF8-5G9 and further
1 comprises a constant region derived from the heavy chain
of human IgG4. The CDR-grafted light chain comprises a
variable region containing FR regions derived from human
antibody RE1 and CDRs derived from murine monoclonal
5 antibody TF8-5G9 and further comprises a constant region
derived from human IgG4 kappa chain. Nucleic acids
encoding the heavy and light chains were constructed by
assembling the variable regions from synthetic
nucleotides, amplifying the assembled variable regions
10 by PCR, purifying the amplified nucleic acids, and
ligating the nucleic acid encoding the variable region
into a vector containing a nucleic acid encoding the
appropriate human constant region.

The sequences of representative nucleic acids
15 encoding CDR-grafted heavy and light chains are
presented as nucleotides 1-2360 of SEQ ID NO:15 and
nucleotides 1-759 of SEQ ID NO:20, respectively.

The nucleic acid sequence encoding a preferred
heavy chain (nucleotides 1-2360 of SEQ ID NO:15) is
20 designated the TF8HCDR20 gene. The nucleic acid
sequence contains the following regions: 5' EcoRI
restriction site (nucleotides 1-6); Kozak sequence
(nucleotides 7-15); start codon and leader sequence
(nucleotides 16-72); CDR-grafted variable region
25 (nucleotides 73-423); human IgG4 CH1 domain (nucleotides
424-717); human IgG4 intron 2 (nucleotides 718-1110);
human IgG4 hinge (nucleotides 1111-1146); human IgG4
intron 3 (nucleotides 1147-1267); human IgG4 CH2 domain
(nucleotides 1268-1594); human IgG4 intron 4
30 (nucleotides 1595-1691); human IgG4 CH3 domain
(nucleotides 1692-2012); 3' untranslated region

(nucleotides 2013-2354); 3' BamHI end spliced to BclI site of expression vector (nucleotides 2355-2360).

The nucleic acid sequence encoding a preferred light chain gene (nucleotides 1-759 of SEQ ID NO:20) is designated the TF8LCDR3 gene. The nucleic acid sequence 5 contains the following regions: 5' EcoRI restriction site (nucleotides 1-5); Kozak sequence (nucleotides 6-8); start codon and leader sequence (nucleotides 9-68); CDR-grafted variable region (nucleotides 69-392); human kappa constant region (nucleotides 393-710); 3' 10 untranslated region (nucleotides 711-753); 3' BamHI end spliced to BclI site of expression vector (nucleotides 754-759).

The foregoing preferred sequences can be modified by the ordinarily skilled artisan to take into 15 account degeneracy of the genetic code, and to make additions, deletions, and conservative and nonconservative substitutions that result in a maintenance of the function of the nucleic acid, i.e. that it encodes a heavy or light chain of a CDR-grafted 20 antibody capable of inhibiting human tissue factor. Restriction sites and sequences that facilitate transcription and translation may be altered or substituted as necessary depending upon the vector and host system chosen for expression.

25 Suitable expression vectors and hosts for production of the CDR-grafted antibodies of the present invention are known to the ordinarily skilled artisan. The expression vectors contain regulatory sequences, such as replicons and promoters, capable of directing 30 replication and expression of heterologous nucleic acids sequences in a particular host cell. The vectors may

also contain selection genes, enhancers, signal
1 sequences, ribosome binding sites, RNA splice sites,
polyadenylation sites, transcriptional terminator
sequences, and so on. The vectors may be constructed by
conventional methods well-known in the art, or obtained
5 from commercial sources. The expression vectors
preferably have convenient restriction sites at which
the nucleic acids encoding the antibody chains of the
invention are inserted. Myeloma expression vectors in
which antibody gene expression is driven by the human
10 cytomegalovirus promoter-enhancer or are particularly
preferred.

Expression vectors containing a nucleic acid
encoding the CDR-grafted heavy chain under the control
of a suitable promoter and expression vectors containing
15 a nucleic acid encoding the CDR-grafted light chain
under the control of a suitable promoter are
cotransfected into a suitable host cell. In another
embodiment, nucleic acids encoding both heavy and light
chains are provided in a single vector for transfection
20 of a suitable host cell.

Suitable host cells or cell lines for
expression of the CDR-grafted antibodies of the present
invention include bacterial cells, yeast cells, insect
cells, and mammalian cells such as Chinese hamster ovary
25 (CHO) cells, COS cells, fibroblast cells and myeloid
cells. Mammalian cells are preferred. CHO, COS and
myeloma cells are particularly preferred. Myeloma cells
are preferred for establishing permanent CDR-grafted
antibody producing cell lines. Expression of antibodies
30 in myeloma cells, bacteria, and yeast is reviewed by

Sandhu (1992) Critical Reviews in Biotechnology 12:437.

1 Expression in mammalian cells is reviewed by Owen et al.
Transfection of host cells by the expression
vectors containing nucleic acids encoding the CDR-
grafted heavy and light chains can be accomplished by
5 methods well-known to one of ordinary skill in the art.
Such methods include, for example, calcium chloride
transfection, calcium phosphate transfection,
lipofection and electroporation. Suitable culture
methods and conditions for the production of the CDR-
10 grafted antibodies are likewise well-known in the art.
The CDR-grafted antibodies can be purified by
conventional methods, including ammonium sulfate
precipitation, affinity chromatography, gel
electrophoresis, and the like. The ability of the CDR-
15 grafted antibodies to bind to and inhibit human tissue
factor can be assessed by the in vitro assays described
above.

The CDR-grafted antibodies of the present
invention have a variety of utilities. For example, the
20 antibodies are capable of binding to human tissue factor
and thus are useful in assays for human tissue factor
from body fluid samples, purification of human tissue
factor, and so on.

The CDR-grafted antibodies of the present
25 invention are capable of inhibiting human tissue factor.
Human tissue factor is well-known to be an essential
element in the human coagulation cascade. The ability
of the antibodies of the present invention to disrupt
the coagulation cascade is demonstrated by in vitro
30 assays in which the antibodies prevent factor X
activation. Accordingly, the present antibodies are

useful in the attenuation of coagulation. The present
1 invention thus provides a method of attenuation of
coagulation comprising administering a therapeutically
effective amount of CDR-grafted antibody capable of
inhibiting human tissue factor to a patient in need of
5 such attenuation.

Numerous thrombotic disorders are
characterized by excessive or inappropriate coagulation
and are effectively treated or prevented by
administration of agents that interfere with the
10 coagulation cascade. Accordingly, the present invention
further provides a method of treatment or prevention of
a thrombotic disorder comprising administering a
therapeutically effective amount of a CDR-grafted
antibody capable of inhibiting human tissue factor to a
15 patient in need of such treatment or prevention. In a
preferred embodiment, the thrombotic disorder is
intravascular coagulation, arterial restenosis or
arteriosclerosis. The antibodies of the invention may be
used in combination with other antibodies or therapeutic
20 agents.

A therapeutically effective amount of the
antibodies of the present invention can be determined by
the ordinarily skilled artisan with regard to the
patient's condition, the condition being treated, the
25 method of administration, and so on. A therapeutically
effective amount is the dosage necessary to alleviate,
eliminate, or prevent the thrombotic disorder as
assessed by conventional parameters. For example, a
therapeutically effective dose of a CDR-grafted antibody
30 of the present invention may be from about 0.1 mg to
about 20 mg per 70 kg of body weight. A preferred

dosage is about 1.0 mg to about 5 mg per 70 kg of body weight.

A patient in need of such treatment is a patient suffering from a disorder characterized by inappropriate or excessive coagulation, or a patient at 5 risk of such a disorder. For example, anticoagulant therapy is useful to prevent postoperative venous thrombosis, and arterial restenosis following balloon angioplasty.

The CDR-grafted antibodies of the present 10 invention are useful in the same manner as comparable therapeutic agents, and the dosage level is of the same order of magnitude as is generally employed with those comparable therapeutic agents. The present antibodies may be administered in combination with a 15 pharmaceutically acceptable carrier by methods known to one of ordinary skill in the art.

Another embodiment of the present invention is directed to a pharmaceutical composition comprising a 20 least one CDR-grafted antibody capable of inhibiting human tissue factor and further comprising a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying 25 agents, and the like. The use of such media and agents for pharmaceutically active substances is well-known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. 30 Supplementary active ingredients can also be incorporated into the compositions.

The antibodies can be administered by well-known routes including oral and parenteral, e.g., intravenous, intramuscular, intranasal, intradermal, subcutaneous, and the like. Parenteral administration and particularly intravenous administration is preferred. Depending on the route of administration, the pharmaceutical composition may require protective coatings.

The pharmaceutical forms suitable for injectionable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the ultimate solution form must be sterile and fluid. Typical carriers include a solvent or dispersion medium containing, for example, water buffered aqueous solutions (i.e., biocompatible buffers), ethanol, polyol such as glycerol, propylene glycol, polyethylene glycol, suitable mixtures thereof, surfactants or vegetable oils. The antibodies may be incorporated into liposomes for parenteral administration. Sterilization can be accomplished by an art-recognized techniques, including but not limited to, addition of antibacterial or antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid or thimersal. Further, isotonic agents such as sugars or sodium chloride may be incorporated in the subject compositions.

Production of sterile injectable solutions containing the subject antibodies is accomplished by incorporating these antibodies in the required amount in the appropriate solvent with various ingredients enumerated above, as required, followed by

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sterilization, preferably filter sterilization. To
1 obtain a sterile powder, the above solutions are vacuum-
dried or freeze-dried as necessary.

The following examples further illustrate the
present invention.

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EXAMPLE 11 Isolation and Sequencing of TF8-5G9
 Light Chain (LC) and Heavy Chain (HC)

Two DNA libraries were generated from oligo 5 (dT)-primed TF8-5G9 hybridoma RNA utilizing standard molecular biology procedures as described by Sambrook et al. The cDNA was cloned into the Librarian II plasmid vector from Invitrogen (San Diego, CA), and the libraries were screened for cDNA clones encoding murine 10 IgG HC and LC. A full-length cDNA clone for the heavy chain could not be isolated, despite the construction of two independent libraries. A random primed TF8-5G9 cDNA library was generated to obtain the missing 5' sequence 15 of the heavy chain. Consequently, the heavy chain cDNA was in two pieces: a 5' clone of 390 nucleotides and a 3' clone of 1392 nucleotides. The two HC clones overlap by 292 nucleotides.

The HC and LC clones were completely sequenced by the dideoxy chain termination method of Sanger et al. 20 (1977) Proc. Natl. Acad. Sci. USA 74:5463. To verify the variable region sequence, sequence was obtained from PCR-amplified cDNA that had been synthesized from total TF8-5G9 hybridoma RNA. Total TF8-5G9 hybridoma RNA was isolated by the guanidinium thiocyanate method of 25 Chrigwin et al. (1970) Biochemistry 18:5294. cDNA was synthesized using the Perkin Elmer (Norwalk, CT) GeneAmp RNA Polymerase Chain Reaction (PCR) kit with an oligo (dT) primer. Components of the same kit were used in the PCR to amplify the LC and HC variable regions using 30 primers based on the sequence that had been obtained for the cDNA clones. The amplified variable region

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fragments were gel-purified and sequenced according to
1 the method of Tracy et al. (1991) BioTechniques 11:68 on
a Model 373A Applied Biosystems, Inc. (Foster City, CA)
automated fluorescent DNA sequencer. The sequence for
TF8-5G9 LC and HC obtained from RNA amplification and
5 the sequence obtained from the cDNA clones agreed. The
TF8-5G9 HC variable region sequence with protein
translation is shown in Figure 1 and SEQ ID NO:1, and
that for the LC is shown in Figure 2 and SEQ ID NO:3.

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EXAMPLE 2

1 Chimeric LC and HC Expression Vector Construction

In order to test the binding activity of the CDR-grafted anti-TF LC and HC individually, mouse-human 5 chimeric TF8-5G9 LC and HC were constructed. This allowed the CDR-grafted LC to be tested for TF binding ability in combination with the chimeric HC, and the CDR-grafted HC to be tested in combination with the chimeric LC.

10 Primers were designed to amplify the TF8-5G9 LC variable region using as template cDNA clones in the Librarian II vector. The 5' primer was designed with an EcoRI site while the 3' primer was designed with a NarI site. PCR was used to amplify the LC variable region, 15 generating a 433 bp fragment with a 5'EcoRI end and 3'NarI end. The fragment included the signal sequence from the TF8-5G9 LC cDNA clone but incorporated a 2 base change in the arginine codon immediately following the ATG start codon. This change retained the arginine 20 residue but made the sequence conform to the Kozak consensus sequence in order to potentially improve translation of the LC mRNA. The PCR amplified LC variable region fragment was digested with EcoRI and NarI restriction enzymes and purified by electrophoresis 25 on a 2% Nusieve, 1% Seakem agarose gel (FMC Bio Products, Rockland, ME).

The DNA was extracted from the gel slice and purified by the Geneclean (Bio 101, La Jolla, CA) procedure. The full-length chimeric TF8-5G9 LC gene was 30 generated by cloning this DNA into the EcoRI and NarI sites of a pSP73 vector (Promega, Madison, WI) which

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1 contains the human kappa constant region. The gene was
1 isolated from the pSP73 vector by EcoRI digestion and
subcloned into the EcoRI site of the pSG5 mammalian cell
expression vector (Stratagene Cloning Systems, La Jolla,
CA).

5 The chimeric TF8-5G9 HC gene was assembled in
a manner similar to that of the chimeric LC. Since
there was no full-length HC cDNA isolated from the
Librarian II vector cDNA libraries, the HC variable
region fragment that was generated by the PCR from total
10 TF8-5G9 hybridoma cell RNA was used as the template.
Primers which incorporated an EcoRI site at the 5' end
and a SacI site at the 3' end were used in the PCR to
generate a 430 bp fragment which contained the TF8-5G9
HC Kozak sequence, start codon, signal sequence, and
15 variable region. This fragment was digested with the
restriction enzymes EcoRI and SacI, and gel-purified
using the same procedure that was used with the chimeric
LC construction.

20 The full-length TF8-5G9 chimeric HC gene was
constructed by cloning the variable region fragment into
the EcoRI and SacI sites of the pSG5 expression vector
containing the human IgG4 constant region.

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EXAMPLE 31 **Design and Construction of the
CDR-Grafted Heavy and Light Chain Genes**

The variable region domains of the CDR-grafted
5 HC and LC genes were designed with an EcoRI overhang at
the 5' end followed by a Kozak sequence to improve
antibody expression. The leader sequences were derived
from the heavy and light chains of the murine monoclonal
antibody B72.3 (Whittle et al. (1987) Protein
10 Engineering 1:499). The 3' end of the variable regions
were designed to have overhangs which allowed for
splicing to the appropriate human constant region DNA.

In the initially designed CDR-grafted TF8-5G9
heavy and light chains the CDRs were derived from murine
15 TF8-5G9 sequence while the frameworks were derived
primarily from human antibody sequence. The human
antibody KOL (Schmidt et al.) was used for the heavy
chain frameworks, while the human antibody dimer (Epp et
al.) was used for the light chain frameworks.

20 Several criteria were used to select murine
framework residues in the design of the TF8-5G9 CDR-
grafted heavy and light chain variable regions.
Framework residues which, at a particular position, are
idiosyncratic to TF8-5G9 were retained as murine
25 sequence with the assumption that they contributed to
its unique binding characteristics. TF8-5G9 murine
residues were also retained at framework positions where
they were in agreement with the human consensus sequence
but where the corresponding residues in KOL or REI were
30 idiosyncratic. Residues that are part of antibody loop
canonical structures such as residue 71 (numbering

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according to Kabat et al.) of the heavy and light chains 1 were also retained as murine sequence. Framework residues that form loops such as residues 26-30 of the HC were kept as TF8-5G9 murine sequence at positions where the murine sequence differed from the human.

5 Residues known to directly influence the conformation of CDRs, such as 48 and 49 immediately preceding CDR2 of the HC, were also retained as murine sequence.

The amino acid sequence of the variable region for the initially designed CDR-grafted TF8-5G9 HC,

10 TF8HCDR1, is shown in SEQ ID NO:11. Murine residues were retained at framework positions 6, 17, 23, 24, 28, 29, 30, 48, 49, 68, 71, 73, 78 88 and 91. The CDR-grafted HC variable region was attached to a human IgG4 constant region.

15 The amino acid sequence of the variable region for the initially designed CDR-grafted TF8-5G9 LC, TF8LCDR1, is shown in SEQ ID NO:12. Murine residues were retained at framework positions 39, 41, 46 and 105. The CDR-grafted LC variable region was attached to a 20 human kappa constant region.

The variable region for the CDR-grafted HC and LC described above were each assembled from 13 synthetic oligonucleotides which were synthesized by Research Genetics, Inc., Huntsville, AL. These oligonucleotides 25 ranged in length from 42 to 80 bases, and encoded both variable region strands. When the 6 complementary oligonucleotide pairs were annealed, the overhangs generated were 17 to 24 bases in length. These oligonucleotide pairs were combined, annealed at their 30 complementary overhangs, and ligated to give the final full length double-stranded variable regions.

The HC variable region oligonucleotides were 1 assembled into a 452 bp fragment which contains a 5' EcoRI site and a 3' SacI site. The polymerase chain reaction was used to amplify this fragment. The resulting amplified DNA was purified on a 2% Nusieve, 1% 5 Seakem agarose gel (FMC). The appropriate size band of DNA was excised and the DNA was recovered by the Geneclean (Bio 101) procedure. The fragment was then digested with EcoRI and SacI, and purified again by the Geneclean method. This HC variable region fragment with 10 EcoRI and SacI ends was cloned into the EcoRI and SacI sites of the pSport-1 vector (GIBCO-BRL Life Technologies, Gaithersburg, MD). DNA from several clones was isolated and sequenced to verify proper variable region assembly. All clones had unexpected 15 base changes. One clone with the fewest base changes (two mismatches at bases 133 and 140) was selected to be corrected by site-directed mutagenesis according to Kunkel (1985) Proc. Natl. Acad. Sci. USA 82:488. Briefly, CJ236 (ung-, dut-) competent cells (Invitrogen 20 Corporation, San Diego, CA) were transformed with the pSport vector containing the CDR-grafted HC variable region with the two base mismatch. Single-stranded, uridine-incorporated DNA templates were purified from phage following M13 helper phage (Stratagene Cloning 25 Systems) infection of the transformed cells. Mutagenesis oligos containing the desired base changes were synthesized on an Applied Biosystems Model 380B DNA synthesizer. The mutagenesis oligos were annealed to the template DNA, and T7 DNA Polymerase and T4 DNA 30 Ligase (MutaGene InVitro Mutagenesis Kit, Bo-Rad Laboratories, Richmond, CA) were used to incorporate the

oligo into a newly synthesized DNA strand. DH5 α
1 competent cells (GIBCO-BRL Life Technologies) were
transformed with the double-stranded DNA. The original
uridine-incorporated strand is destroyed while the newly
synthesized strand containing the mutagenesis oligo is
5 replicated. Phagemid DNA was prepared from the
resulting mutagenesis clones and the variable regions
were sequenced to identify the clones which had
incorporated the desired changes. The corrected HC
EcoRI/SacI variable region fragment was excised from the
10 pSport vector, purified and ligated into the EcoRI/SacI
sites of a pSG5 vector containing the human IgG4
constant region. This resulted in the generation of a
full-length humanized TF8-5G9 HC gene, TF8HCDR1, in the
pSG5 COS cell expression vector. The vector was
15 designated pSG5TF8HCDR1.

The CDR-grafted TF8-5G9 LC variable region was
also amplified by the PCR from the assembled synthetic
oligonucleotides into a 433 bp fragment which contained
a 5' EcoRI site and a 3' NarI site. This fragment was
20 purified as described above for the HC, digested with
EcoRI and NarI and purified by the Geneclean procedure.
This fragment was cloned into the EcoRI and NarI sites
of a pSG5 vector which contains the human kappa constant
region. This resulted in the generation of a full-
25 length humanized TF8-5G9 LC gene, TF8LCDR1, in the pSG5
COS cell expression vector. Seven clones were
sequenced, and one was found to have the desired CDR-
grafted LC sequence. The vector was designated
pSQ5TF8LCDR1.

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EXAMPLE 41 **Expression of the CDR-Grafted
Heavy and Light Chain Genes in COS Cells**

The transient expression of antibody genes in
5 COS-1 cells provides a rapid and convenient system to
test antibody gene expression and function. COS-1 cells
were obtained from the American Type Culture Collection
(CRL 1650) and cultured in Dulbecco's Modified Eagle
Medium (DMEM, from GIBCO BRL Life Technologies) with 10%
10 fetal calf serum. The pSG5TF8HCDR1 expression factor
was cotransfected into COS cells with the pSG5 chimeric
LC expression vector using the DEAE-Dextran method
followed by DMSO shock as described by Lopata *et al.*
(1984) Nucleic Acids Res. 14:5707. After 4 days of
15 culture, media was harvested from the wells and examined
for antibody expression levels.

Antibody levels were determined by an ELISA-
based assembly assay. Plates were coated with a goat
anti-human Fc specific antibody. Various dilutions of
20 the COS cell supernatant containing secreted antibody
were added, incubated for one hour, and washed. A
horseradish peroxidase-linked goat anti-human kappa
chain antibody was added, incubated for one hour at room
temperature, and washed. Substrate for the horseradish
25 peroxidase was added for detection. Antibody levels in
the COS cell media were found to be nearly undetectable
for the TF8HCDR1 x chimeric LC. Upon closer examination
of the TF8HCDR1 variable region sequence, it was found
that an unexpected base change, which had occurred
30 during the site-directed mutagenesis process described
in Example 3, introduced a stop codon into framework 4

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of the TF8HCDR1 gene. This substitution was corrected
1 by site-directed mutagenesis as described above.

Thorough sequencing of the variable region confirmed
that the correction was made with no additional changes
introduced. Upon transfection of this corrected
5 TF8HCDR1 gene with the chimeric LC, reasonable
expression levels were obtained.

COS cells which had been co-transfected with
the CDR-grafted LC expression vector, pSGTF8LCDR1, and
either the chimeric HC or TF8HCDR1, produced antibody at
10 reasonable levels. Antibody levels in COS cell
supernatants ranged from 0.5 μ g to 10.0 μ g per ml.

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EXAMPLE 5

1 Binding of the CDR-Grafted TF8-5G9 to Tissue Factor

An ELISA was used to determine the ability of the CDR-grafted TF8-5G9 antibody, TF8HCDR1 x TF8LCDR1, 5 to bind to tissue factor. Tissue factor was immobilized on a microtiter plate. The test COS cell supernatant, containing the CDR-grafted antibody, was added to the well, incubated for one hour at room temperature. Following three washes with PBS/Tween, a goat anti-human 10 kappa chain polyclonal antibody conjugated to horseradish peroxidase was added, incubated for one hour at room temperature and washed. Substrate for the horseradish peroxidase was added for detection. The positive control was the TF8-5G9 chimeric antibody. The 15 CDR-grafted TF8-5G9 antibody was able to bind to tissue factor to a degree comparable to the chimeric TF8-5G9 antibody (Figure 3, solid symbols).

The ability of the humanized antibody to compete with murine TF8-5G9 for binding to tissue factor 20 was also examined. Varying amounts of COS cell supernatant containing the test CDR-grafted antibody and a fixed amount of murine TF8-5G9 were added simultaneously to wells coated with tissue factor. Binding was allowed to occur for one hour at room 25 temperature. The wells were washed three times with PBS/Tween. A goat anti-human kappa chain antibody conjugated to horseradish peroxidase was added, incubated for one hour at room temperature and washed. Substrate for the horseradish peroxidase was added for 30 detection. The positive antibody competed as well as

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the chimeric antibody with murine TF8-5G9 for binding to
1 TF.

These data indicate that the initially
designed CDR-grafted antibody, TF8HCDR1 x TF8LCDR1, was
approximately as active as the chimeric TF8-5G9 in
5 binding to TF and competing with the murine antibody for
binding to TF.

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EXAMPLE 61 **Construction and Characterization**
 of Additional CDR-Grafted Heavy Chains

Upon examination of the molecular structure of 5 murine TF8-5G9, framework residues at positions 27, 68, 73 and 78 were found to lie on the antibody surface and had no discernible contact with the CDRs. These framework residues were of murine sequence in TF8HCDR1 but were changed to the human KOL sequence in various 10 combinations to generate a series of CDR-grafted heavy chains with framework residue variations. The changes were made by the process of site-directed mutagenesis as described in Example 3. Each CDR-grafted heavy chain version was expressed in COS cells in combination with 15 the CDR-grafted LC, TF8LCDR1, and tested for its ability to bind TF and compete with murine TF8-5G9 for binding. Every version of the CDR-grafted heavy chain in combination with TF8LCDR1 was shown to bind TF with an affinity comparable to chimeric TF8-5G9. Every CDR- 20 grafted HC in combination with TF8LCDR1 was able to compete with murine TF8-5G9 for binding to TF to a degree comparable to the chimeric antibody.

Changes in sequence from murine to human for HC framework positions 6, 7, 68, 73 and 78 did not 25 adversely affect the antigen binding ability of the antibody. The CDR-grafted HC version which had human sequence at all of these positions, and thus was the most humanized HC, was TF8HCDR20.

The complete sequence of the TF8HCDR20 gene 30 was determined. The DNA sequence is shown as a 2360 bp EcoRI/BamHI insert with protein translation in the

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pEe6TF8HCDR20 expression vector in Figure 4 and SEQ ID
1 NO:15.

The essential regions of the gene are as
follows:

	Nucleotide #	Region
5	1-6	5' <u>Eco</u> RI restriction site
	7-15	Kozak sequence
	16-72	Start codon and leader sequence
	73-423	CDR-grafted variable region
	424-717	Human IgG4 CH1 domain
10	718-1110	Human IgG4 intron 2
	1111-1146	Human IgG4 hinge
	1147-1267	Human IgG4 intron 3
	1268-1594	Human IgG4 CH2 domain
	1595-1691	Human IgG4 intron 4
15	1692-2012	Human IgG4 CH3 domain
	2013-2354	3' untranslated region
	2355-2360	3' <u>Bam</u> HI end spliced to <u>Bcl</u> I site of the expression vector

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EXAMPLE 71 Construction and Characterization
 of Additional CDR-Grafted Light Chains

The initially designed CDR-grafted LC,
5 TF8LCDR1, contained four framework residues from the
murine TF8-5G9 sequence. At two of these positions, 39
and 105, the human REI framework sequence is unique to
REI; however, the murine TF8-5G9 LC sequence is in
agreement with the human consensus sequence. The other
10 two murine framework residues, trp41 and thr46, are
unique to TF8-5G9. Several versions of the CDR-grafted
LC were generated in which the sequence at these four
positions were changed from the murine to the human REI
in various combinations. These changes were made by
15 site-directed mutagenesis. Each version of the CDR-
grafted LC was expressed in COS cells in combination
with the CDR-grafted HC, TF8HCDR20, and tested for
ability to bind tissue factor and compete with murine
TF8-5G9 for binding. Every version of the CDR-grafted
20 LC, in combination with TF8HCDR20, was shown to bind TF
with an affinity comparable to TF8-5G9. Also every CDR-
grafted LC version, in combination with TF8HCDR20, was
able to compete with murine TF8-5G9 for binding to TF in
a manner comparable to the chimeric TF8-5G9 control.

25 Changes in sequence from murine to human for
LC framework positions 39, 41, 46 and 105 did not
adversely effect the ability of the antibody to
recognize antigen. The CDR-grafted LC of choice was
TF8LCDR3, where murine TF8-5G9 sequence was used at
30 positions 39 and 105 because these are in agreement with

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the human consensus sequence. The preferred CDR-grafted
1 TF8-5G9 antibody is TF8HCDR20 x TF8LCDR3.

The complete sequence of the TF8LCDR3 gene was
determined and is shown as a 759 bp EcoRI-BamHI insert
with protein translation in the pEel2TF8LCDR3 expression
5 vector in Figure 5 and SEQ ID NO:17. The essential
regions of the gene are as follows:

	Nucleotide #	Region
	1-5	5' <u>Eco</u> RI restriction site
	6-8	Kozak sequence
10	9-68	Start codon and leader sequence
	69-392	CDR-grafted variable region
	393-710	Human kappa constant region
	711-753	3' untranslated region
15	754-759	3' <u>Bam</u> HI end spliced to <u>Bcl</u> I site of the expression vector

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EXAMPLE 81 CDR-Grafted TF8-5G9 Antibody TF8HCDR20 x TF8LCDR3
Inhibits Human Tissue Factor

5 The binding of the CDR-grafted TF8-5G9 antibody, TF8HCDR20 x TF8LCDR3, to TF was assessed as described in Example 5 and was found to be comparable to that of the chimeric TF8-5G9 as illustrated in Figure 6. The ability of the CDR-grafted TF8-5G9 to compete with the murine antibody for binding to TF is comparable to 10 that of the chimeric TF8-5G9 as shown in Figure 7.

15 An in vitro assay was used to measure the level of inhibition of factor X activation by the CDR-grafted TF8-5G9 antibody. In this assay, TF forms an active proteolytic complex with factor VII. This complex then converts factor X to factor Xa by proteolysis. The activated Xa enzymatically cleaves a substrate, Spectrozyme FXa, which releases a chromogen. The level of chromogen, as detected by optical density, 20 is an indication of factor X activation due to TF-factor VIIa activity.

The following reaction mixtures were prepared in 12 x 75 mm borosilicate glass tubes.

25 25 μ l TBS (50 mM Tris, pH 7.4, 150 mM NaCl)
15 μ l 20 mM CaCl₂/1% bovine serum albumin
25 (BSA)
30 20 μ l human placental tissue factor solution (prepared by reconstituting one vial of Thromborel S, Curtin Matheson Scientific #269-338 with 4.0 ml dH₂O and diluting 1:10 in TBS)

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30 μ l Factor VII (Enzyme Research Labs #HFVII
1 1007 at 237.66 ng/ml in TBS)
30 μ l TBS or TF8-5G9 or TF8MCDR20 x TF8LCDR3
at 1.18 μ g/ml or as indicated in Fig. 8
The reaction mixtures were incubated at 37°C
5 for ten minutes before the addition of Factor X. (In
some cases the reaction mixture was preincubated for
five minutes before addition of Factor VII or antibody,
followed by a ten minute incubation before addition of
Factor X.) Thirty μ l of Factor X solution (Enzyme
10 Research Labs, DHFX 330, 247.38 μ g/ml TBS) was added and
the mixture was incubated at 37°C for three minutes.
Factor X activation was terminated by pipetting 40 μ g of
reaction mixture into 160 μ l of stop buffer (50 mM Tris,
pH 7.4, 100 mM EDTA, 150 mM NaCl) in 96 well microtiter
15 plates. Each tube of reaction mixture was pipetted into
three microtiter wells. Fifty μ l of Spectrozyme FXa
substrate (American Diagnostica #222, 1 μ M/ml TBS) was
added to each well. OD₄₀₅ was read on a Molecular
Devices kinetic plate reader with readings taken every
20 twenty seconds for ten minutes. Factor X activity was
recorded as mOD/minute, and enzyme velocities over the
linear portion of the reaction curve were compared to
determine inhibition of factor X activation by the anti-
TF antibodies.
25 As shown in Figure 8, the CDR-grafted TF8-5G9
antibody is approximately as effective as the murine
TF8-5G9 in inhibiting factor X activation. This
indicates that the CDR-grafted TF8-5G9 is functionally
active.

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EXAMPLE 91 **Construction of the CDR-Grafted Heavy
and Light Chain Myeloma Expression Vectors**

For the purpose of establishing a permanent
5 CDR-grafted antibody-producing cell line, the TF8HCDR20
and TF8LCDR3 genes were subcloned into myeloma cell
expression vectors. The heavy chain TF8HCDR20 was
subcloned into the EcoRI and BclI sites of the pEe6hCMV-
BglII myeloma expression vector described by Stephens *et*
10 *al.* (1989) Nucleic Acids Res. 17:7110 to produce
pEe6TF8HCDR20. The light chain TF8LCDR3 was subcloned
into the EcoTI and BclI sites of the pEe12 myeloma
expression vector to produce pEe12TF8LCDR3. The heavy
and light chain expression vectors are illustrated in
15 Figures 9 and 10, respectively. In both vectors
antibody gene transcription was driven by the human
cytomegalovirus (hCMV) promoter-enhancer, which lies
directly 5' to the multiple cloning site. The
polyadenylation signal sequence lies 3' to the multiple
20 cloning site and signals the termination of
transcription. Each vector contains the β -lactamase
gene to allow for ampicillin selection in E. coli. The
pEe12 vector contains a glutamine synthetase cDNA gene
under the transcriptional control of the SV40 early
25 promoter. Glutamine synthetase allows for myeloma cell
transfectants to be selected in glutamine-free media.
Myeloma cells are devoid of glutamine synthetase
activity and are dependent on a supply of glutamine in
the culture media. Cells which have been transfected
30 with the pEe12 vector, containing the glutamine

synthetase gene, are able to synthesize glutamine from 1 glutamate and can survive in the absence of glutamine.

The pEe6TF8HCDR20 expression vector is a 7073 bp plasmid whose DNA sequence is shown in Figure 4 and SEQ ID NO:15. The coding regions of the TF8HCDR20 gene are 5 translated. The essential regions of this vector are described below:

1. Nucleotides #1-2360: The TF8HCDR20 CDR-grafted HC gene is described in Example 6. The HC gene was inserted as an EcoRI/BamHI fragment into the EcoRI/BclI sites of the pEe6hCMV-BglII vector.
- 10 2. Nucleotides #2361-2593: This region encodes the SV40 early gene polyadenylation signal (SV40 nucleotides 2770-2537), which acts as a transcriptional terminator. This fragment is flanked by a 5' BclI site and a 3' BamHI site. The 3' BamHI end of the heavy chain gene was spliced to the 5' BclI site of the polyadenylation signal, thus eliminating both sites.
- 15 3. Nucleotides #2594-3848: This region is a BamHI-BglI fragment from pBR328 (nucleotides 375-2422) but with a deletion between the SalI and AvaI sites (pBR328 nucleotides 651-1425) following the addition of a SalI linker to the AvaI site. This region contains the Col E1 bacterial origin of replication.
- 20 4. Nucleotides #3849-4327: This is a BglI-XmnI fragment site from the β -lactamase gene of pSP64 (Promega Corporation, Madison, WI). This gene provides ampicillin resistance to bacteria transformed with this vector.
- 25 5. Nucleotides #4328-4885: This is an XmnI-HindIII fragment of the ColE1 based plasmid pCT54 described by Emtage et al. (1983) Proc. Natl. Acad. Sci. USA.

1 80:3671. The HindIII site was converted
to a BglII site by the addition of a
linker following the addition of the hCMV
promoter described below.

5 6. Nucleotides #4886-7022: These
nucleotides encode the Pst-1m fragment of
human cytomeglovirus (hCMV) strain AD 169
described by Greenway *et al.* (1982) Gene
18:355 containing the region coding for
the hCMV middle intermediate early
promoter. This Pst-1m fragment was
cloned into the HindIII site of pEe6hCMV
by addition of oligonucleotides of the
10 following sequence to either end of the
fragment:

5' GTCACCGTCCTTGACACGA 3'

3' ACGTCAGTGGCAGGAACGTGCTTCGA 5'

15 The resulting 2100 bp fragment was
inserted such that the promoter directed
transcription towards the EcoRI site of
pEe6hCMV. The oligonucleotide above
served to recreate the complete 5'
untranslated sequence of the hCMV-MIE
gene the added irrelevant sequence at the
very 5' end of the fragment. The HindIII
20 site at the 5' end was subsequently
converted to a BglII site by the addition
of a further linker.

25 7. Nucleotides #7023-7073: The pSP64
polylinker with the BamHI and SaII sites
removed.

25 The pEe12TF8LCDR3 expression vector is a 7864
bp plasmid whose DNA sequence is shown in Figure 5 and
SEQ ID NO:17. The coding regions of the TF8LCDR3 gene
are translated. The essential regions of this
expression vector are described below:

30 1. Nucleotides #1-759: The TF8LCDR3 CDR-
grafted LC gene is described in Example
7. The gene was inserted as an

1 4. Nucleotides #5737-7864: This region is identical to the hCMV promoter and pSP64 polylinker encoded by nucleotides 4886-7073 of the pEe6TF8HCDR20 vector described above (regions 6 and 7).

5 For the purpose of ensuring that both the pEe6TF8HCDR20 and pEe12TF8LCDR3 vectors co-transfected myeloma cells, the vectors were joined in linear concatamers. Both the pEe6TF8HCDR20 and pEe12TF8LCDR3 vectors were digested at the unique SalI site. The SalI linearized pEe6TF8HCDR20 vector was phosphatased at its 10 5' ends to prohibit ligation of two pEe6TF8HCDR20 vectors onto each other. This phosphatased HC vector was ligated in a 2:1 molar ratio to the SalI linearized pEe12TF8LCDR3. The resulting concatamers were most likely of the following composition:

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<u>Sal</u> I	<u>Sal</u> I	<u>Sal</u> I	<u>Sal</u> I
pEe6TF8HCDR20	pEe12TF8LCDR3	pEe6TF8HCDR20	

20 This concatamerized DNA was extracted with phenol and chloroform, and precipitated with ammonium acetate and ethanol. The DNA precipitate was resuspended in distilled water to a concentration of 1 μ g/ μ L and used to transfect myeloma cells.

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EXAMPLE 10

1 Development of NSO Expression Cell Lines

Stably transformed cell lines expressing the humanized TF8-5G9 antibody were prepared by transfecting 5 CDR-grafted heavy and light chain expression vectors into NSO mouse myeloma cells. Selection of transfected cells was carried out using the dominant selectable marker gene, glutamine synthetase (GS).

The NSO mouse myeloma cell line, obtained from 10 Celltech, Ltd., is a subclone derived from NS-1 and does not express intracellular light chains. These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with added glutamine and 10% fetal bovine serum (FBS). To prepare for transfection, the cells were 15 harvested in mid-log phase of the growth cycle, centrifuged for 5 minutes, washed with phosphate buffered saline (PBS), centrifuged again, and the cell pellet was resuspended in 2.2 mL of PBS. The final cell concentration was 2.18×10^7 mL. Cells were maintained 20 on ice during the entire procedure.

The DNA to be transfected (pEe12TF8LCDR3 x pEe6TF8HCDR20) was prepared as a concatamer as described in Example 9. The DNA and NSO cells were added to a 0.4 cm BioRad Gene Pulser cuvette in the following order:

25 40 μ L (40 μ g) DNA concatamer
 320 μ L double distilled water
 40 μ L 10 x PBS
 400 μ L NSO cells (8.72×10^6 cells)

Transfection was performed by electroporation 30 following a protocol provided by Celltech, Ltd. In this procedure, the cells and DNA in PBS buffer were exposed

to a brief, high voltage pulse of electricity causing
1 transient micropores to form on the cell membrane. DNA
transfer takes place through these openings. To prepare
for electroporation, the suspension of NS0 cells and DNA
was gently mixed and incubated on ice for 5 minutes.

5 The cuvette was placed in a BioRad Gene Pulser and given
2 consecutive electrical pulses at settings of 3 μ F
(capacitance) and 1.5V (voltage). Following
electroporation, the cuvette was returned to the ice for
5 minutes. The suspension was then diluted in prewarmed
10 growth medium and distributed into seven 96-well plates.
Control plates containing cells electroporated without
DNA were also prepared at the same time to measure the
presence of spontaneous mutants. Plates were placed in
a 37°C incubator with 5% CO₂.

15 Glutamine synthetase, encoded by the GS gene,
is an enzyme that converts glutamate to glutamine. NS0
cells require glutamine for growth due to inadequate
levels of endogenous GS gene expression. In the DNA
concatamer, this gene is located on the pEel2TF8LCDR3
20 vector. Transfected cells which incorporate the GS gene
become glutamine-independent. Cells not integrating the
GS gene into their genome would remain glutamine-
dependent and would not survive in glutamine-free
medium. Approximately 18 hours post electroporation,
25 all plates were fed with glutamine-free selection medium
and returned to the incubator until viable colonies
appeared.

30 Approximately 3 weeks after transfection,
distinct macroscopic colonies were observed. These were
screened for expression of the intact humanized antibody
using the assembly ELISA as described in Example 5.

Tissue culture supernatants from wells containing 1 colonies were screened at a 1:10 dilution. Positive wells showing activity greater than the 25 ng/mL standard were subcultured and expanded for further analysis.

5 For selection of high producers, antibody production was quantitated after a 96 hour growth period. Tissue culture flasks were seeded with 2×10^5 cells/mL in 10 mL of selection medium and incubated at 37°C, 5% CO₂, for 96 hours. At the end of that time 10 period, an aliquot was taken to determine cell concentration and antibody titer. Evaluation of antibody production was calculated as $\mu\text{g/mL}$ and pg/cell/96 hours. The highest producers from this transfection were:

15	<u>Cell Line</u>	<u>$\mu\text{g/mL}$</u>	<u>pg/cell/96 hour</u>
	2B1	26.3	24.3
	3E11	27.6	59.9
	4G6	30.2	41.9

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EXAMPLE 111 **CDR Grafted Antibody TF8HCDR20 x TF8LCDR3**
 Inhibits Tissue Factor In Vivo

CDR grafted antibody TF8HCDR20 x TF8LCDR3 was
 5 compared to murine antibody TF8-5G9 for its ability to
 protect rats from experimentally induced disseminated
 intravascular coagulation (DIC). In the DIC model, rats
 are challenged with human thromboplastin (a crude tissue
 extract containing TF activity), resulting in fibrinogen
 10 consumption and death. Pretreatment of rats with anti-
 TF antibody was demonstrated to protect rats from
 fibrinogen consumption and death as follows.

Human thromboplastin was prepared as described
 in U.S. Patent 5,223,427. Saline control or 30 μ /ml of
 15 TF8-5G9 or CDR-grafted antibody was injected through the
 tail vein of rats, followed by injection of
 thromboplastin equivalent to 200 ng of recombinant TF.
 Clotting times were determined at T=0 and T=1 minute as
 a measure of fibrinogen concentration. Clotting times
 20 are proportional to fibrinogen concentration, with a 60
 second clotting time corresponding to an 80% reduction
 in fibrinogen concentration. Clotting times of greater
 than 60 seconds cannot be accurately measured and were
 recorded as 60 seconds.

25 Survivability and clotting times for three
 representative studies are shown below.

Survivors

	Study	Controls	TF8-5G9	CDR-grafted Ab
30	1	0/8	5/8	6/8
	2	0/8	4/7	7/8
	3	0/8	8/8	3/7

		<u>Clotting Times</u>					
		<u>Controls</u>					
		<u>Study #1</u>		<u>Study #2</u>		<u>Study #3</u>	
		<u>T=0</u>		<u>T=1</u>		<u>T=0</u>	
1	Study #1 <u>T=0</u> <u>T=1</u>	Study #2 <u>T=0</u> <u>T=1</u>	Study #3 <u>T=0</u> <u>T=1</u>				
16	>60	18	>60	19	>60		
16	>60	18	>60	21	>60		
5	16 >60	18 >60	18 >60				
17	>60	18	>60	19	>60		
15	>60	16	>60	18	54		
16	>60	18	>60	18	>60		
16	>60	17	>60	18	>60		
16	>60	17	>60	18	>60		
10	<u>Clotting Times</u> <u>Murine TF8-5G9</u>						
		<u>Study #1</u>		<u>Study #2</u>		<u>Study #3</u>	
		<u>T=0</u>		<u>T=1</u>		<u>T=0</u>	
15	16 36	18 34	19	28			
15	41	18 36	18	29			
15	33	18 >60	19	29			
15	31	17 >60	18	29			
15	>60	18 50	18	28			
16	>60	17 34	19	40			
16	33	17 34	19	40			
16	33	18 31	19	34			
16	>60		19	>60			
20	<u>Clotting Times</u> <u>CDR-grafted TF8-5G9</u>						
		<u>Study #1</u>		<u>Study #2</u>		<u>Study #3</u>	
		<u>T=0</u>		<u>T=1</u>		<u>T=0</u>	
25	16 >60	17 >60	21	>60			
16	>60	17 33	18	34			
16	>60	18 32	17	>60			
22	37	18 >60	20	35			
16	32	17 32	17	58			
15	>60	18 31	18	33			
16	>60	17 31	18	31			
16	>60	16 32					
30							

Twenty-three of the twenty-four control rats
1 had clotting times of greater than 60 seconds indicating
that virtually all untreated rats were consuming more
than 80% of their fibrinogen. Both the CDR-grafted and
murine antibody treated rats had similar clotting times
5 at one minute of 44.5 and 40 seconds. Further, only six
of the murine antibody treated rats and nine of the CDR-
grafted antibody treated rats had clotting times in
excess of 60 seconds. Accordingly, both the murine and
CDR-grafted antibodies were able to neutralize TF and
10 thus protect rats from fibrinogen consumption and death.

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SEQUENCE LISTING

1

(1) GENERAL INFORMATION:

(i) APPLICANT: Joliffe, Linda K.
Zivin, Robert A.
Pulito, Virginia L.

5

(ii) TITLE OF INVENTION: CDR-GRAFTED ANTI-TISSUE FACTOR
ANTIBODIES AND METHODS OF USE THEREOF

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

15

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 07-JUN-1995
(C) CLASSIFICATION:

20

(viii)- ATTORNEY/AGENT INFORMATION:
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30

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(2) INFORMATION FOR SEQ ID NO:1:

1 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1489 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

5 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 11..1391

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

10	GGTCCTTACA ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val 1 5 10	49
15	GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu 15 20 25	97
20	CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly 30 35 40 45	145
25	TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu 50 55 60	193
30	CAG GGC CTG GAG TGG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr 65 70 75	241
35	ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr 80 85 90	289
40	TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp 95 100 105	337
45	ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr 110 115 120 125	385

	TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC	433
1	Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro 130 135 140	
	CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC	481
	Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser 145 150 155	
5	ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG	529
	Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val 160 165 170	
	ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC	577
	Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe 175 180 185	
10	CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT	625
	Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Val Thr 190 195 200 205	
	GTC CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC	673
	Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala 210 215 220	
	CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT	721
	His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp 225 230 235	
15	TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC	769
	Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val 240 245 250	
	TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT	817
	Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr 255 260 265	
20	CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG	865
	Pro Lys Val Thr Cys Val Val Asp Ile Ser Lys Asp Asp Pro Glu 270 275 280 285	
	GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG	913
	Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln 290 295 300	
25	ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT	961
	Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser 305 310 315	

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	GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA	1009
1	Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys 320 325 330	
	TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC	1057
	Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile 335 340 345	
5	TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA	1105
	Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro 350 355 360 365	
	CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTG AAC TGC ATG	1153
	Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met 370 375 380	
10	ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TGG CAG TGG AAT	1201
	Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn 385 390 395	
	GGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA	1249
	Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr 400 405 410	
	GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC	1297
	Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn 415 420 425	
15	TGG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG	1345
	Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu 430 435 440 445	
	CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA T	1391
	His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys 450 455 460	
20	GATCCCAGTG TCCTTGGAGC CCTCTGGTCC TACAGGACTC TGACACCTAC CTCCACCCCT	1451
	CCCTGTATAA ATAAAGCACC CAGCACTGCC TTGGACCC	1489

(2) INFORMATION FOR SEQ ID NO:2:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 460 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Gly
1 5 10 15

5 Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg
20 25 30

Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile
35 40 45

Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
50 55 60

10 Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp
65 70 75 80

Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr Ser Ser Asn
85 90 95

Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val
100 105 110

15 Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln
115 120 125

Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val
130 135 140

Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr
145 150 155 160

20 Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr
165 170 175

Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val
180 185 190

Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
195 200 205

25 Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala
210 215 220

1 Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys
225 230 235 240
Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe
245 250 255
Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val
260 265 270
5 Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe
275 280 285
Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro
290 295 300
Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro
305 310 315 320
10 Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val
325 330 335
Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
340 345 350
Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys
355 360 365
15 Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met Ile Thr Asp
370 375 380
Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro
385 390 395 400
Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser
405 410 415
20 Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala
420 425 430
Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His
435 440 445
His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
450 455 460

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(2) INFORMATION FOR SEQ ID NO:3:

1 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 937 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

5 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 5..706

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

10	GGAC ATG CGG GCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TGG TTT Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe 1 5 10 15	49
15	CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met 20 25 30	97
20	TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln 35 40 45	145
25	GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser 50 55 60	193
30	CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG GCA GAT GGG GTC CCA Pro Lys Thr Leu Ile Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro 65 70 75	241
35	TCA AGA TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTA ACC ATC Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile 80 85 90 95	289
40	AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His 100 105 110	337
45	GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC Gly Glu Ser Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile Asn 115 120 125	385

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1	AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu 130 135 140	433
5	CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe 145 150 155	481
10	TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg 160 165 170 175	529
15	CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser 180 185 190	577
20	ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu 195 200 205	625
25	CGA CAT AAC AGC TAT ACC TGT GAG CCC ACT CAC AAG ACA TCA ACT TCA Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser 210 215 220	673
30	CCC AAT GTC AAG AGC TTC AAC AAG AAT GAG TGT TAGAGACAAA GGTCCTGAGA Pro Asn Val Lys Ser Phe Asn Lys Asn Glu Cys 225 230	726
35	CGCCACCACC AGCTCCCCAG CTCCATCCTA TCTTCCCTTC TAAGGTCTTG GAGGCTTCCC CACAAGCGAC CTACCACTGT TGCAGTGCTC CAAACCTCCT CCCCACCTCC TTCTCCTCCT CCTCCCTTTC CTTGGCTTTT ATCATGCTAA TATTTGCAGA AAATATTCAA TAAAGTGAGT CTTTGCACTT GAAAAAAA AAAAAAAA A	786 846 906 937

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 234 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

1 Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe Pro
 1 5 10 15

Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
 20 25 30

Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
 5 35 40 45

Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser Pro
 50 55 60

Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser
 65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
 10 85 90 95

Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His Gly
 100 105 110

Glu Ser Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile Asn Arg
 115 120 125

Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
 15 130 135 140

Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
 145 150 155 160

Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
 165 170 175

Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
 20 180 185 190

Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
 195 200 205

His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
 210 215 220

Asn Val Lys Ser Phe Asn Lys Asn Glu Cys
 25 225 230

(2) INFORMATION FOR SEQ ID NO:5:

1 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Asp Tyr Met His
1 5

10 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Lys Pro Lys Phe Gln
1 5 10 15
Gly

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

1 Asp Asn Ser Tyr Tyr Phe Asp Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:8:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Ala Ser Gln Asp Ile Arg Lys Tyr Leu Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Tyr Ala Thr Ser Leu Ala Asp
1 5

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(2) INFORMATION FOR SEQ ID NO:10:

1 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Leu Gln His Gly Glu Ser Pro Tyr Thr
1 5

10 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Leu Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

20 Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
50 55 60

Gln Gly Arg Phe Ser Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Phe
65 70 75 80

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1 Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
100 105 110
Val Thr Val Ser Ser
115

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(2) INFORMATION FOR SEQ ID NO:12:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

15 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ala Pro Lys Thr Leu Ile
35 40 45
Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
20 Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
85 90 95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
100 105

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(2) INFORMATION FOR SEQ ID NO:13:

1 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

10 Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
50 55 60

Gln Gly Arg Phe Thr Ile Ser Ala Asp Asn Ser Lys Asn Thr Leu Phe
65 70 75 80

15 Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
100 105 110

20 Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:14:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

1 (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

5

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
20 25 30

10

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
100 105

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(2) INFORMATION FOR SEQ ID NO:15:

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7073 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 61..717

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1111..1146

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1 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1268..1594

1 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1692..2012

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAATTCGCCT CCACCATGGA ATGGAGCTGG GTCTTCTCT TCTTCTGTC AGTAAC	60
GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA GGA GGA GTA GTA	108
Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val	
1 5 10 15	
CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT AGT GGA TTC AAT	156
10 Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn	
20 25 30	
ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT CCT GGA AAA GGA	204
Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly	
35 40 45	
CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT AAC ACG ATA TAT	252
Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr	
15 50 55 60	
GAT CCC AAG TTC CAA GGA AGA TTC ATA ATT TCT GCA GAC AAC TCT AAG	300
Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys	
65 70 75 80	
AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT GAG GAT ACA GCA	348
Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala	
85 90 95	
20 GTC TAC TTT TGT GCT AGA GAT AAC AGT TAT TAC TTC GAC TAC TGG GGC	396
Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly	
100 105 110	
CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC AAG GGC CCA TCC	444
Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser	
115 120 125	
25 GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC	492
Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala	
130 135 140	

	GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG	540
	Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val	
1	145 150 155 160	
	TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT	588
	Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala	
	165 170 175	
5	GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG	636
	Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val	
	180 185 190	
	CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC GTA GAT CAC	684
	Pro Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His	
	195 200 205	
10	AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGTGAGAGGC CAGCACAGGG	737
	Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val	
	210 215	
	CAGGGAGGGT GTCTGCTGGA ACCCAGGCTC AGCCCTCCCTG CCTGGACGCA CCCCCGGCTGT	797
	GCAGCCCCAG CCCAGGGCAG CAAGGCATGC CCCATCTGTC TCCTCACCCG GAGGCCTCTG	857
	ACCACCCCCAC TCATGCTCAG GGAGAGGGTC TTCTGGATTT TTCCACCAGG CTCCGGGCAG	917
15	CCACAGGCTG GATGCCCTA CCCCAGGCC CGCGCATACA GGGGCAGGTG CTGCGCTCAG	977
	ACCTGCCAAG AGCCATATCC GGGAGGACCC TGCCCTGAC CTAAGCCCAC CCCAAAGGCC	1037
	AAACTCTCCA CTCCCTCAGC TCAGACACCT TCTCTCCTCC CAGATTCGAG TAACTCCCAA	1097
	TCTTCTCTCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA	1146
	Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro	
	1 5 10	
20	GGTAAGCCAA CCCAGGCCTC GCCCTCCAGC TCAAGGCAGG ACAGGTGCC TAGAGTAGCC	1206
	TGCATCCAGG GACAGGCCCC AGCCGGGTGC TGACGCATCC ACCTCCATCT CTTCCTCAGC	1266
	A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC CCC CCA AAA	1312
	Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys	
	1 5 10 15	
25	CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG	1360
	Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val	
	20 25 30	

1	GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr 35 40 45	1408
5	GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu 50 55 60	1456
10	CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC ATG CAC Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His 65 70 75	1504
15	CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys 80 85 90 95	1552
20	GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys 100 105	1594
25	GGTGGGACCC ACGGGGTGCG AGGGCCACAT GGACAGAGGT CAGCTGGCC CACCCCTCTGC CCTGGGAGTG ACCGCTGTGC CAACCTCTGT CCCTACAA GGG CAG CCC CGA GAG CCA Gly Gln Pro Arg Glu Pro 1 5	1654 1709
30	CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln 10 15 20	1757
35	GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala 25 30 35	1805
40	GTG GAG TGG GAG AGT AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr 40 45 50	1853
45	CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu 55 60 65 70	1901
50	ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA GTC TCC Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Val Ser 75 80 85	1949

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	GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser	1997
1	90 95 100	
	CTG TCT CTG GGT AAA TGAGTGCCAG GGCCGGCAAG CCCCCGCTCC CCGGGCTCTC Leu Ser Leu Gly Lys	2052
	105	
5	GGGGTCGCGC GAGGATGCTT GGCACGTACC CCGTCTACAT ACTTCCCAGG CACCCAGCAT GGAAATAAAAG CACCCACAC TGCCCTGGGC CCCTGTGAGA CTGTGATGGT TCTTTCCACG	2112
	GGTCAGGCCG AGTCTGAGGC CTGAGTGACA TGAGGGAGGC AGAGCGGGTC CCACTGTCCC	2172
	CACACTGGCC CAGGCTGTGC AGGTGTGCCT GGGCCACCTA GGGTGGGGCT CAGCCAGGGG	2232
	CTGCCCTCGG CAGGGTGGGG GATTTGCCAG CGTGGCCCTC CCTCCAGCAG CAGGACTCTA	2292
10	10 GAGGATCATA ATCAGCCATA CCACATTTGT AGAGGTTTA CTTGCTTTAA AAAACCTCCC ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT GTTGTGTTA ACTTGTGTTAT	2352
	TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT	2412
	TTTTTCACTG CATTCTAGTT GTGGTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG	2472
	GATCCTCTAC GCCGGACGCA TCGTGGCCGG CATCACCGGC GCCACAGGTG CGGTTGCTGG	2532
15	CGCCTATATC GCCGACATCA CCGATGGGA AGATCGGGCT CGCCACTTCG GGCTCATGAG	2592
	CGCTTGTGTTG GGCAGTGGGTA TGGTGGCAGG CCCGTGGCCG GGGGACTGTT GGGCGCCATC	2652
	TCCTTGCATG CACCATTCCCT TGCGGCGGCG GTGCTCAACG GCCTCAACCT ACTACTGGC	2712
	TGCTTCCTAA TGCAGGAGTC GCATAAGGGA GAGCGTCGAC CTCGGGCCGC GTTGTGGCG	2772
20	20 TTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG	2832
	CGCTCTCCTG TTCCGACCCCT GCCGCTTACC GGATACCTGT CCGCCTTCT CCCTTCGGGA	2892
	AGCGTGGCGC TTTCTCAATG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC	2952
	TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCG ACCGCTGCAC CTTATCCGGT	3012
25	AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT	3072
		3132
		3192
		3252

	GGTAACAGGA TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG	3312
1	CCTAACTACG GCTACACTAG AAGGACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT	3372
	ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAAC AAACCACCCG TGTTAGCGGT	3432
	GGTTTTTTG TTTGCAAGCA GCAGATTACG CGCAGAAAAA AAGGATCTCA AGAAGATCCT	3492
5	TTGATCTTTT CTACGGGTC TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTG	3552
	GTCATGAGAT TATCAAAAAG GATCTTCACC TAGATCCTT TAAATTAAAA ATGAAGTTT	3612
	AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT	3672
	GAGGCACCTA TCTCAGCGAT CTGTCATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC	3732
	GTGTAGATAA CTACGATAACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATAACCG	3792
10	CGAGACCCAC GCTCACCGGC TCCAGATTAA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC	3852
	GAGCGCAGAA GTGGTCCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG	3912
	GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCAGA ACGTTGTTGC CATTGCTACA	3972
	GGCATCGTGG TGTCACGCTC GTCGTTGGT ATGGCATCAT TCAGCTCCGG TTCCCAACGA	4032
15	TCAAGGCCAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTTCGGTCT	4092
	CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG	4152
	CATAATTCTC TTACTGTCAT GCCATCCGTA AGATGCTTT CTGTGACTGG TGAGTACTCA	4212
	ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTGCCCG GGCCTCAACA	4272
	CGGGATAATA CCGCGCCACA TAGCAGAACT TTAAAGTGC TCATCATTGG AAAACGTTCT	4332
20	TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT	4392
	CGTGCACCCA ACTGATCTTC AGCATCTTT ACTTTCACCA GCGTTCTGG GTGAGCAAAA	4452
	ACAGGAAGGC AAAATGCCGC AAAAAGGGGA ATAAGGGGA CACGGAAATG TTGAATACTC	4512
	ATACTCTTCC TTTTCATAA TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA	4572
25	TACATATTG AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGGCGAC ATTTCCCCGA	4632
	AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAAACCTA TAAAAATAGG	4692

	CGTATCACGA GGCCCTGATG GCTCTTGCG GCACCCATCG TTCTGTAATGT TCCGTGGCAC	4752
1	CGACGACAAC CCTCAAGAGA AAATGTAATC ACACCTGGCTC ACCTTCGGGT GGGCCTTCCT	4812
	GCGTTTATAA GGAGACACTT TATGTTTAAG AAGGTTGGTA AATTCCCTGCG GGCTTTGGCA	4872
	GCCAAGCTAG AGATCTCTAG CTTCGTGTCA AGGACGGTGA CTGCAGTGAA TAATAAAATG	4932
5	TGTGTTGTC CGAAATACGC GTTTGAGAT TTCTGTCGCC GACTAAATTC ATGTCGCGCG	4992
	ATAGTGGTGT TTATCGCCGA TAGAGATGGC GATATTGGAA AAATCGATAT TTGAAAATAT	5052
	GGCATATTGA AAATGTCGCC GATGTGAGTT TCTGTGTAAC TGATATCGCC ATTTTCCAA	5112
	AAGTGATTTT TGGGCATAACG CGATATCTGG CGATAGCGCT TATATCGTTT ACGGGGGATG	5172
	GCGATAGACG ACTTTGGTGA CTTGGCGAT TCTGTGTGTC GCAAATATCG CAGTTTCGAT	5232
10	ATAGGTGACA GACGATATGA GGCTATATCG CCGATAGAGG CGACATCAAG CTGGCACATG	5292
	GCCAATGCAT ATCGATCTAT ACATTGAATC AATATTGGCC ATTAGCCATA TTATTCATTG	5352
	GTTATATAGC ATAAATCAAT ATTGGCTATT GGCCATTGCA TACGTTGTAT CCATATCATA	5412
	ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT TGATTATTGA	5472
15	CTAGTTATTA ATAGTAATCA ATTACGGGT CATTAGTTCA TAGCCCATAT ATGGAGTTCC	5532
	GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCCAGCCAT	5592
	TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC	5652
	AATGGGTGGA GTATTTACGG TAAACTGCC ACCTGGCAGT ACATCAAGTG TATCATATGC	5712
	CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCAGT	5772
20	ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA CGTATTAGTC ATCGCTATTA	5832
	CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT GACTCACGGG	5892
	GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT GTTTGGCAC CAAATCAAC	5952
	GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC GGTAGGCGTG	6012
	TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC GCCTGGAGAC	6072
25	GCCATCCACG CTGTTTGAC CTCCATAGAA GACACCGGGA CCGATCCAGC CTCCGGGCC	6132

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	GGGAACGGTG CATTGGAACG CGGATTCCCC GTGCCAAGAG TGACGTAAGT ACCGCCTATA	6192
1	GAGTCTATAG GCCCACCCCC TTGGCTTCTT ATGCATGCTA TACTGTTTT GGCTTGGGGT	6252
	CTATACACCC CCGCTTCCTC ATGTTATAGG TGATGGTATA GCTTAGCCTA TAGGTGTGGG	6312
	TTATTGACCA TTATTGACCA CTCCCCTATT GGTGACGATA CTTTCCATT A CTAATCCATA	6372
5	ACATGGCTCT TTGCCACAAAC TCTCTTTATT GGCTATATGC CAATACACTG TCCTTCAGAG	6432
	ACTGACACGG ACTCTGTATT TTTACAGGAT GGGGTCTCAT TTATTATTAA CAAATTCA	6492
	TATACAACAC CACCGTCCCC AGTGCCCGCA GTTTTTATTAA AACATAACGT GGGATCTCCA	6552
	CGCGAATCTC GGGTACGTGT TCCGGACATG GGCTCTTCTC CGGTAGCGGC GGAGCTTCTA	6612
	CATCCGAGCC CTGCTCCCAT CCCTCCAGCG ACTCATGGTC GCTCGGCAGC TCCTTGCTCC	6672
10	TAACAGTGGA GGCCAGACTT AGGCACAGCA CGATGCCAC CACCACCA GTGCCGCACA	6732
	AGGCCGTGGC GGTAGGGTAT GTGTCTGAAA ATGAGCTCGG GGAGCGGGCT TGCACCGCTG	6792
	ACGCATTGG AAGACTTAAG GCACGGCAG AAGAAGATGC AGGCAGCTGA GTTGGTTGTGT	6852
	TCTGATAAGA GTCAGAGGTA ACTCCCGTTG CGGTGCTGTT AACGGTGGAG GGCAGTGTAG	6912
15	TCTGAGGAGT ACTCGTTGCT GCGCGCGCG CCACCAAGACA TAATAGCTGA CAGACTAAC	6972
	GACTGTTCCCT TTCCATGGGT CTTTCTGCA GTCACCGTCC TTGACACGAA GCTTGGGCTG	7032
	CAGGTCGATC GACTCTAGAG GATCGATCCC CGGGCGAGCT C	7073

(2) INFORMATION FOR SEQ ID NO:16:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

1 Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Val Val
 1 5 10 15

Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn
 20 25 30

Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
 5 35 40 45

Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr
 50 55 60

Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys
 65 70 75 80

Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala
 10 85 90 95

Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
 115 120 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
 15 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
 20 180 185 190

Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val
 210 215

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(2) INFORMATION FOR SEQ ID NO:17:

1 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
1 5 10 15

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
20 25 30

20 Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
35 40 45

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Gln
50 55 60

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His Gln
65 70 75 80

25 Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
85 90 95

30

35

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
100 105
1

(2) INFORMATION FOR SEQ ID NO:19:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

10 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
1 5 10 15
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
50 55 60
Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
65 70 75 80
Asn Val Phe Ser Val Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
20 100 105

(2) INFORMATION FOR SEQ ID NO:20:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7864 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

1 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 9..711

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

5	AATTCACCAT GGGTGTGCCA ACTCAGGTAT TAGGATTACT GCTGCTGTGG CTTACAGATG	60
	CAAGATGTGA TATCCAAATG ACACAATCTC CTTCTTCTCT AAGTGCTTCT GTCGGAGATA	120
	GAGTAACAAT TACATGTAAG GCGAGTCAGG ACATTAGAAA GTATTTAAC TGTTATCAGC	180
	AAAAACCTGG GAAGGCTCCT AAGCTACTGA TTTATTATGC AACAGTTG GCAGATGGAG	240
	TACCTTCTAG ATTTTCTGGT TCTGGCTCTG GAACAGACTA CACATTACAA ATTCTTCTC	300
10	TCCAAACCTGA GGACATTGCT ACATACTACT GCCTACAACA TGGTGAGAGT CCGTATAACAT	360
	TTGGACAAGG AACAAAACAA GAGATCACAA GAACTGTTGC GGCGCCGTCT GTCTTCATCT	420
	TCCCGCCATC TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTTGTGTGC CTGCTGAATA	480
	ACTTCTATCC CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA	540
15	ACTCCCAGGA GAGTGTACA GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA	600
	CCCTGACGCT GAGCAAAGCA GACTACGAGA AACACAAAGT CTACGCCCTGC GAAGTCACCC	660
	ATCAGGGCCT GAGCTCGCCC GTCACAAAGA GCTTCAACAG GGGAGAGTGT TAGAGGGAGA	720
	AGTGCCCCCA CCTGCTCCTC AGTCCAGCC TGGGGATCAT AATCAGCCAT ACCACATTTG	780
	TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA	840
20	TGAATGCAAT TGTTGTTGTT AACTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA	900
	ATAGCATCAC AAATTCACA AATAAACAT TTTTTCACT GCATTCTAGT TGTGGTTGT	960
	CCAAACTCAT CAATGTATCT TATCATGTCT GGATCCTCTA CGCCGGACGC ATCGTGGCCG	1020
	GCATCACCGG CGCCACAGGT GCGGTTGCTG GCGCCTATAT CGCCGACATC ACCGATGGGG	1080
25	AAGATCGGGC TCGCCACTTC GGGCTCATGA GCGCTTGTGTT CGGCGTGGGT ATGGTGGCAG	1140

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	GCCCCGTGGCC	GGGGGACTGT	TGGGCGCCAT	CTCCTTGCAT	GCACCATTCC	TTGCAGCGGC	1200
1	GGTGCTCAAC	GGCCTCAACC	TACTACTGGG	CTGCTTCCTA	ATGCAGGAGT	CGCATAAGGG	1260
	AGAGCGTCGA	CCTCGGGCCG	CGTTGCTGGC	GTTCCTCCAT	AGGCTCCGCC	CCCCTGACGA	1320
	GCATCACAAA	AATCGACGCT	CAAGTCAGAG	GTGGCGAAC	CCGACAGGAC	TATAAAGATA	1380
5	CCAGGCCTTT	CCCCCTGGAA	GCTCCCTCGT	GCGCTCTCCT	GTTCGACCC	TGCCGCTTAC	1440
	CGGATAACCTG	TCCGCCTTTC	TCCCTTCGGG	AAGCGTGGCG	CTTCTCAAT	GCTCACGCTG	1500
	TAGGTATCTC	AGTTCGGTGT	AGGTGTTCG	CTCCAAGCTG	GGCTGTGTGC	ACGAACCCCC	1560
	CGTTCAAGCCC	GACCGCTGCG	CCTTATCCGG	TAACTATCGT	CTTGAGTCCA	ACCCGGTAAG	1620
	ACACGACTTA	TCGCCACTGG	CAGCAGCCAC	TGGTAACAGG	ATTAGCAGAG	CGAGGTATGT	1680
10	AGGCAGGTGCT	ACAGAGTTCT	TGAAGTGGTG	GCCTAACTAC	GGCTACACTA	GAAGGACAGT	1740
	ATTTGGTATC	TGCGCTCTGC	TGAAGCCAGT	TACCTTCGGA	AAAAGAGTTG	GTAGCTTTG	1800
	ATCCGGCAAA	CAAACCACCG	CTGGTAGCGG	TGGTTTTTT	GTTCGAAGC	AGCAGATTAC	1860
	GGCGAGAAAA	AAAGGATCTC	AAGAAGATCC	TTTGATCTT	TCTACGGGGT	CTGACGCTCA	1920
15	GTGGAACGAA	AACTCACGTT	AAGGGATTTT	GGTCATGAGA	TTATCAAAAA	GGATCTTCAC	1980
	CTAGATCCTT	TTAAATTAAA	AATGAAGTTT	TAAATCAATC	TAAAGTATAT	ATGAGTAAAC	2040
	TTGGTCTGAC	AGTTACCAAT	GCTTAATCAG	TGAGGCACCT	ATCTCAGCGA	TCTGTCTATT	2100
	TCGTTCATCC	ATAGTTGCCT	GAATCCCCGT	CGTGTAGATA	ACTACGATAC	GGGAGGGCTT	2160
	ACCATCTGGC	CCCAGTGCTG	CAATGATACC	GCGAGACCCA	CGCTCACCGG	CTCCAGATT	2220
20	ATCAGCAATA	AACCAGCCAG	CCGGAAGGGC	CGAGCGCAGA	AGTGGCCTG	CAACTTTATC	2280
	CGCCTCCATC	CAGTCTATTA	ATTGTTGCCG	GGAAAGCTAGA	GTAAGTAGTT	CGCCAGTTAA	2340
	TAGTTTGCAG	AACGTTGTTG	CCATTGCTAC	AGGCATCGTG	GTGTCACGCT	CGTCGTTGG	2400
	TATGGCTTCA	TTCAGCTCCG	GTTCCCAACG	ATCAAGGCAG	GTTACATGAT	CCCCCATGTT	2460
25	GTGCAAAAAA	GCGGTTAGCT	CCTTCGGTCC	TCCGATCGTT	GTCAGAAGTA	AGTTGGCCGC	2520
	AGTGTATCA	CTCATGGTTA	TGGCAGCACT	GCATAATTCT	CTTACTGTCA	TGCCATCCGT	2580

	AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG	2640
1	GCGACCGAGT TGCTCTGCC CGCGTCAAC ACGGGATAAT ACCGCGCCAC ATAGCAGAAC	2700
	TTTAAAAGTG CTCATCATTG GAAAACGTTT TTCGGGGCGA AAACTCTCAA GGATCTTACC	2760
	GCTGTTGAGA TCCAGTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTT	2820
5	TACTTCACC AGCGTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG	2880
	AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTCAAT ATTATTGAAG	2940
	CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATT GAATGTATTT AGAAAAATAA	3000
	ACAAATAGGG GTTCCCGCGA CATTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT	3060
	TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG AGGCCCTGAT GGCTCTTGC	3120
10	GGCACCCATC GTTCGTAATG TTCCGTGGCA CCGAGGACAA CCCTCAAGAG AAAATGTAAT	3180
	CACACTGGCT CACCTTCGGG TGGCCTTTC TCGCTTATA AGGAGACACT TTATGTTAA	3240
	GAAGGTTGGT AAATTCCCTG CGGCTTGGC AGCCAAGCTA GAGATCCGGC TGTGGAATGT	3300
	GTGTCAGTTA GGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GCCAGAAGTA TGCAAAGCAT	3360
	GCATCTCAAT TAGTCACCAA CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC	3420
15	TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC	3480
	CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTAA TTTTATTTAT GCAGAGGCCG	3540
	AGGCCGCCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTT GGAGGCCCTAG	3600
	GCTTTGCAA AAAGCTAGCT TGGGGCCACC GCTCAGAGCA CCTTCCACCA TGGCCACCTC	3660
20	AGCAAGTTCC CACTTGAAACA AAAACATCAA GCAAATGTAC TTGTGCCTGC CCCAGGGTGA	3720
	GAAAGTCCAA GCCATGTATA TCTGGGTTGA TGGTACTGGA GAAGGACTGC GCTGCAAAAC	3780
	CCGCACCCCTG GACTGTGAGC CCAAGTGTGT AGAAGACTTA CCTGAGTGGAA ATTTTGATGG	3840
	CTCTAGTACC TTTCAGTCTG AGGGCTCCAA CAGTGACATG TATCTCAGCC CTGTTGCCAT	3900
	GTTCGGGAC CCCTTCCGCA GAGATCCCAA CAAGCTGGTG TTCTGTGAAG TTTTCAAGTA	3960
25	CAACCGGAAG CCTGCAGAGA CCAATTAAAG GCACTCGTGT AAACGGATAA TGGACATGGT	4020

	GAGCAACCAG CACCCCTGGT TTGGAATGGA ACAGGAGTAT ACTCTGATGG GAACAGATGG	4080
1	GCACCCCTTT GGTTGGCCTT CCAATGGCTT TCCTGGGCC CAAGGTCCGT ATTACTGTGG	4140
	TGTGGGCGCA GACAAAGCCT ATGGCAGGGA TATCGTGGAG GCTCACTACC GCGCCTGCTT	4200
	GTATGCTGGG GTCAAGATTA CAGGAACAAA TGCTGAGGTC ATGCCTGCC AGTGGGAACT	4260
	5 CCAAATAGGA CCCTGTGAAG GAATCCGCAT GGGAGATCAT CTCTGGTGG CCCGTTCAT	4320
5	CTTNCATCGA GTATGTGAAG ACTTTGGGT AATAGCAACC TTTGACCCCA AGCCCATTCC	4380
	TGGGAACTGG AATGGTGCAG GCTGCCATAC CAACTTTAGC ACCAAGGCCA TGCGGGAGGA	4440
	GAATGGTCTG AAGCACATCG AGGAGGCCAT CGAGAAACTA AGCAAGCGGC ACCGGTACCA	4500
	CATTGAGCC TACGATCCCA AGGGGGGCCT GGACAATGCC CGTGGTCTGA CTGGGTTCCA	4560
10	CGAAACGTCC AACATCAACG ACTTTCTGC TGGTGTGCC AATCGCAGTG CCAGCATCCG	4620
	CATTCCCCCG ACTGTGGCC AGGAGAAGAA AGGTTACTTT GAAGACCGCG GCCCCTCTGC	4680
	CAATTGTGAC CCCTTGCAG TGACAGAAGC CATCGTCCGC ACATGCCCTC TCAATGAGAC	4740
	TGGCCACGAG CCCTTCCAAT ACAAAAACTA ATTAGACTTT GAGTGTACTT GAGCCTTCC	4800
	15 TAGTTCATCC CACCCCGCCC CAGAGAGATC TTTGTGAAGG AACCTTACTT CTGTGGTGTG	4860
15	ACATAATTGG ACAAAACTACC TACAGAGATT TAAAGCTCTA AGGTAAATAT AAAATTTTA	4920
	AGTGTATAAT GTGTTAAACT ACTGATTCTA ATTGTTGTG TATTTAGAT TCCAACCTAT	4980
	GGAACTGATG AATGGGAGCA GTGGTGAAT GCCTTTAATG AGGAAAACCT GTTTGCTCA	5040
	GAAGAAATGC CATCTAGTGA TGATGAGGCT ACTGCTGACT CTCAACATTC TACTCCTCCA	5100
20	AAAAAGAAGA GAAAGGTAGA ACACCCCAAG GACTTCCCTT CAGAATTGCT AAGTTTTTG	5160
	AGTCATGCTG TGTTTAGTAA TAGAACTCTT GCTTGCTTGT CTATTACAC CACAAAGGAA	5220
	AAAGCTGCAC TGCTATACAA GAAAATTATG GAAAATATT CTGTAACCTT TATAAGTAGG	5280
	CATAACAGTT ATAATCATAA CATACTGTTT TTTCTTACTC CACACAGGCA TAGAGTGTCT	5340
	GCTATTAATA ACTATGCTCA AAAATTGTGT ACCTTTAGCT TTTTAATTG TAAAGGGTT	5400
25	AATAAGGAAT ATTTGATGTA TAGTGCCTAG ACTAGAGATC ATAATCAGCC ATACCACATT	5460

	TGTAGAGGTT TTACTTCCTT TAAAAAACCT CCCACACCTC CCCCTGAACC TGAAACATAA	5520
1	AATGAATGCA ATTGTTGTTG TTAACTTGTT TATTGCAGCT TATAATGGTT ACAAAATAAG	5580
	CAATAGCATC ACAAAATTCA CAAATAAAGC ATTTTTTCA CTGCATTCTA GTTGTGGTTT	5640
	GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCTCT AGCTTCGTGT CAAGGACGGT	5700
5	GACTGCAGTG AATAATAAAA TGTGTGTTG TCCGAAATAC GCGTTTGAG ATTTCTGTGCG	5760
	CCTACTAAAT TCATGTCGCG CGATAGTGGT GTTTATCGCC GATAGAGATG GCGATATTGG	5820
	AAAAAATCGAT ATTTGAAAAT ATGGCATATT GAAAATGTGCG CCGATGTGAG TTTCTGTGTA	5880
	ACTGATATCG CCATTTTCC AAAAGTGATT TTTGGGCATA CGCGATATCT GGGCGATAGCG	5940
	CTTATATCGT TTACGGGGGA TGGCGATAGA CGACTTTGGT GACTTGGCG ATTCTGTGTC	6000
10	TCGCAAATAT CGCAGTTTCG ATATAGGTGA CAGACGATAT GAGGCTATAT CGCCGATAGA	6060
	GGCGACATCA AGCTGGCACA TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG	6120
	CCATTAGCCA TATTATTCA TGGTTATATA GCATAAAATCA ATATTGGCTA TTGGCCATTG	6180
	CATACGTTGT ATCCATATCA TAATATGTAC ATTTATATTG GCTCATGTCC AACATTACCG	6240
	CCATGTTGAC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT	6300
15	CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA	6360
	CCGCCCCAAG ACCCCCCCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA	6420
	ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTAC GGTAAACTGC CCACTTGGCA	6480
	GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG	6540
20	CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGACT TTCTACTTG GCAGTACATC	6600
	TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGCGT	6660
	GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT	6720
	TTGTTTGGC ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG	6780
	ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TCGTTTAGTG	6840
25	AACCGTCAGA TCGCCTGGAG ACGCCATCCA CGCTGTTTG ACCTCCATAG AAGACACCGG	6900

	GACCGATCCA GCCTCCGCGG CGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG	6960
1	AGTGACGTAA GTACCGCCTA TAGAGTCTAT AGGCCCACCC CCTTGGCTTC TTATGCATGC	7020
	TATACTGTT TTGGCTTCGG GTCTATACAC CCCCCTTCC TCATGTTATA GGTGATGGTA	7080
	TAGCTTAGCC TATAGGTGTG GGTTATTGAC CATTATTGAC CACTCCCCTA TTGGTGACGA	7140
5	TACTTCCAT TACTAATCCA TAACATGGCT CTTTGCCACA ACTCTCTTA TTGGCTATAT	7200
	GCCAATACAC TGTCTTCAG AGACTGACAC GGACTCTGTA TTTTACAGG ATGGGGTCTC	7260
	ATTTATTATT TACAAATTCA CATATACAAC ACCACCGTCC CCAGTGCCCC CAGTTTTAT	7320
	TAAACATAAC GTGGGATCTC CACGCGAATC TCGGGTACGT GTTCCGGACA TGGGCTCTTC	7380
	TCCGGTAGCG GCGGAGCTTC TACATCCGAG CCCTGCTCCC ATGCCTCCAG CGACTCATGG	7440
10	TCGCTCGGCA TCTCCTTGCT CCTAACAGTG GAGGCCAGAC TTAGGCACAG CACGATGCC	7500
	ACCACCAACCA GTGTGCCGCA CAAGGCCGTG GCGGTAGGGT ATGTGTCTGA AAATGAGCTC	7560
	GGGGAGCGGG CTTGCACCGC TGACGCATTG GGAAGACTTA AGGCAGCGGC AGAAGAAGAT	7620
	GCAGGCAGCT GAGTTGTTGT GTTCTGATAA GAGTCAGAGG TAACTCCGT TGCGGTGCTG	7680
15	TTAACCGGTGG AGGGCAGTGT AGTCTGAGCA GTACTCGTTG CTGCCGCCG CGCCACCAGA	7740
	CATAATAGCT GACAGACTAA CAGACTGTTG CTTTCCATGG GTCTTTCTG CAGTCACCGT	7800
	CCTTGACACG AAGCTTGGGC TGCAGGTGCA TCGACTCTAG AGGATCGATC CCCGGGCGAG	7860
	CTCG	7864

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WHAT IS CLAIMED IS:

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1. A CDR-grafted antibody capable of inhibiting human tissue factor wherein the complementarity determining regions (CDRs) are derived 5 from a non-human monoclonal antibody against tissue factor and the framework (FR) and constant (C) regions are derived from one or more human antibodies.

2. The CDR-grafted antibody of Claim 1 wherein said non-human monoclonal antibody is a murine 10 antibody.

3. The CDR-grafted antibody of Claim 2 wherein said murine antibody is TF8-5G9.

4. The CDR-grafted antibody of Claim 1 wherein said CDRs of the heavy chain have the amino acid 15 sequences:

CDR1	DDYMH	(SEQ ID NO:5)
CDR2	LIDPENGNTIYDPKFQG	(SEQ ID NO:6)
CDR3	DNSYYFDY	(SEQ ID NO:7)

and said CDRs of the light chain have the amino acid 20 sequences:

CDR1	KASQDIRKYLN	(SEQ ID NO:8)
CDR2	YATSLAD	(SEQ ID NO:9)
CDR3	LQHGESPYT	(SEQ ID NO:10).

5. The CDR-grafted antibody of Claim 1 25 wherein the FR of the heavy chain is derived from the human antibody KOL.

6. The CDR-grafted antibody of Claim 1 wherein the FR of the light chain is derived from the human antibody REI.

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7. The CDR-grafted antibody of Claim 1
1 wherein the heavy chain variable region has the amino
acid sequence of SEQ ID NO:11.

8. The CDR-grafted antibody of Claim 1 or 7
wherein the light chain variable region has the amino
5 acid sequence of SEQ ID NO:12.

9. The CDR-grafted antibody of Claim 1
wherein the heavy chain variable region has the amino
acid sequence of SEQ ID NO:13.

10. The CDR-grafted antibody of Claim 1 or 9
10 wherein the light chain variable region has the amino
acid sequence of SEQ ID NO:14.

11. The CDR-grafted antibody of Claim 1
wherein the heavy chain constant region is the human
IgG4 constant region.

15 12. The CDR-grafted antibody of Claim 10
wherein the heavy chain constant region is the human
IgG4 constant region.

13. The CDR-grafted antibody of Claim 1
wherein the light chain constant region is the human
20 kappa constant region.

14. The CDR-grafted antibody of Claim 10
wherein the light chain constant region is the human
kappa constant region.

15. CDR-grafted monoclonal antibody TF8HCDR1
25 x TF8LCDR1.

16. CDR-grafted monoclonal antibody TF8HCDR20
x TF8LCDR3.

17. A fragment of the CDR-grafted antibody of
Claim 1 wherein said fragment is capable of inhibiting
30 human tissue factor.

18. The fragment of Claim 17 wherein said
1 fragment is an Fab or F(ab')₂ fragment.

19. A method of making the CDR-grafted
antibody of Claim 1 comprising cotransfected a host
cell with an expression vector comprising a nucleic acid
5 encoding the CDR-grafted antibody heavy chain and an
expression vector comprising a nucleic acid encoding the
CDR-grafted antibody light chain; culturing the
transfected host cell; and recovering said CDR-grafted
antibody.

10 20. A method of making the CDR-grafted
antibody of Claim 1 comprising transfecting a host cell
with an expression vector comprising a nucleic acid
encoding the CDR-grafted antibody heavy chain and a
nucleic acid encoding the CDR-grafted antibody light
15 chain; culturing the transfected host cell; and
recovering said CDR-grafted antibody.

21. The method of Claim 18 or 19 wherein said
nucleic acid encoding the CDR-grafted antibody heavy
chain has the sequence of nucleotides 1-2360 of SEQ ID
20 NO:15.

22. The method of Claim 18 or 19 wherein said
nucleic acid encoding the CDR-grafted light chain has
the sequence of nucleotides 1-759 of SEQ ID NO:17.

23. The method of Claim 19 or 20 wherein said
25 host cell is a bacterial cell, yeast cell, insect cell
or mammalian cell.

24. The method of Claim 23 wherein said
mammalian cell is a CHO cell, COS cell or myeloma cell.

25. The method of Claim 19 wherein said
30 expression vector comprising a nucleic acid encoding the
CDR-grafted antibody heavy chain is pEe6TF8HCDR20.

26. The method of Claim 19 wherein said
1 expression vector comprising a nucleic acid encoding the
CDR-grafted antibody light chain is pEe12TF8LCDR3.

27. A nucleic acid encoding the heavy chain
of the CDR-grafted antibody of Claim 1.

5 28. A nucleic acid encoding the light chain
of the CDR-grafted antibody of Claim 1.

29. The nucleic acid of Claim 27 having the
sequence of nucleotides 1-2360 of SEQ ID NO:15.

30. The nucleic acid of Claim 28 having the
10 sequence of nucleotides 1-759 of SEQ ID NO:17.

31. A method of attenuation of coagulation
comprising administering a therapeutically effective
amount of a CDR-grafted antibody capable of inhibiting
human tissue factor to a patient in need of said
15 attenuation.

32. The method of Claim 31 wherein said CDR-
grafted antibody is TF8HCDR20 x TF84CDR3.

33. A method of treatment or prevention of
thrombotic disorder comprising administering a
20 therapeutically effective amount of a CDR-grafted
antibody capable of inhibiting human tissue factor to a
patient in need of said treatment or prevention.

34. The method of Claim 33 wherein said
thrombotic disorder is intravascular coagulation,
25 arterial restenosis or arteriosclerosis.

35. The method of Claim 33 or 34 wherein said
CDR-grafted antibody is TF8HCDR20 x TF8LCDR3.

36. A pharmaceutical composition comprising
at least one CDR-grafted antibody capable of inhibiting
30 human tissue factor and a pharmaceutically acceptable
carrier.

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37. The pharmaceutical composition of Claim
1 36 wherein said CDR-grafted antibody is TF8HCDR20 x
TF8LCDR3.

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Sequence of the murine TF8-5G9 heavy chain cDNA with protein translation. The essential regions of the cDNA are as follows:

FIG. 1 A

<u>Nucleotides</u>	<u>Region</u>
1-10	5' untranslated region.
11-67	Start codon and leader sequence.
68-418	Variable region.
419-1390	Murine IgG1 constant region.
1391-1489	3' untranslated region.

Sequence Range: 1 to 1489

10	20	30	40	
•	*	*	*	
GGT CCT TAC A ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG				
CCA GGA ATG T TAC TTT ACG TCG ACC CAG TAG AAG AAG GAC TAC CGT CAC				
Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val>				
50	60	70	80	90
*	*	*	*	*
GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG CCT GAG				
CAA TGT CCC CAG TTA AGT CTC TAA GTC GAC GTC GTC AGA CCC CGA CTC				
Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu>				
100	110	120	130	140
*	*	*	*	*
CTT GTC AGG CCA CGG CCC TTA GTC AAG TTG TCC TGC AAA CCT TCT GGC				
GAA CAC TCC GGT CCC CGG AAT CAG TTC AAC AGG ACG TTT CGA AGA CGG				
Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly>				
150	160	170	180	190
*	*	*	*	*
TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTC AAG CAG AGG CCT CAA				
AAG TTG TAA TTT CTC ATG ATA TAC GTG ACC CAC TTC GTC TCC CGA CTT				
Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu>				
200	210	220	230	240
*	*	*	*	*
CAC CGC CTG GAG TCG ATT CGA TTG ATT GAT CCT GAG AAT GGT AAT ACT				
GTC CGG GAC CTC ACC TAA CCT AAC TAA CTA GGA CTC TTA CCA TTA TGA				
Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr>				
250	260	270	280	
*	*	*	*	
ATA TAT GAC CCC AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA				
TAT ATA CTG CGC TTC AAG GTC CCC TTC CGG TCA TAT TGT CGT CTC TGT				
Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr>				
290	300	310	320	330
*	*	*	*	*
TCC TCC AAC ACA GGC TAC CTG CAG CTC ACC AGC AGC CTG ACA TCT GAG GAC				
AGG AGG TTC TGT CGG ATG GAC GTC GAG TCG TCG GAC TGT AGA CTC CTG				
Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp>				

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FIG. 1 B

340 350 360 370 380
 * * * * *
 ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC
 TGA CGG CAG ATA ATG ACA CGA TCT CTA TTG AGC ATG ATG AAA CTG ATG
 Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr>

 390 400 410 420 430
 * * * * *
 TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC
 ACC CCG GTT CCG TGG TGA GAG TGT CAG AGG AGT CCG TTT TGC TGT GGG
 Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro>

 440 450 460 470 480
 * * * * *
 CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT CCT CCC CAA ACT AAC TCC
 GGT AGA CAG ATA GGT GAC CGG GGA CCT AGA CGA CGG GTT TGA TTG AGG
 Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser>

 490 500 510 520
 * * * *
 ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG
 TAC CAC TGG GAC CCT ACG GAC CAG TTC CCG ATA AAG GGA CTC GGT CAC
 Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val>

 530 540 550 560 570
 * * * * *
 ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC
 TGT CAC TGG ACC TTG AGA CCT AGG GAC AGG TCG CCA CAC GTG TGG AAC
 Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe>

 580 590 600 610 620
 * * * * *
 CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT
 CGT CGA CAG GAC GTC AGA CTG GAG ATG TGA GAC TCG TCC AGT CAC TGA
 Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Val Thr>

 630 640 650 660 670
 * * * * *
 GTC CCC TCC ACC ACC TGG CCC ACC GAG ACC GTC ACC TGC AAC GTT GCC
 CAC CGG AGG TCG TCC ACC CGG TCG CTC TGG CAG TCG AGC TTG CAA CGG
 Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala>

 680 690 700 710 720
 * * * * *
 CAC CGG CCC AGC ACC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT
 GTG CGG CGG TCG TCC TTC CAC CTG TTC TTT TAA CAC CGG TCC CTA
 His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp>

 730 740 750 760
 * * * *
 TGT CGT TGT AAC CCT TCC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC
 ACA CCA ACA TTC CGA ACC TAT ACA TGT CAG GGT CTT CAT AGT AGA CAG
 Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val>

 770 780 790 800 810
 * * * * *
 TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT
 AAC TAG AAC CGG CGT TTC CGG TTC CTA CAC GAC TCG TAA TGA GAC TGA
 Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr>

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FIG. 1 C

820 830 840 850 860
 CCT AAG GTC ACG TGT GTT GTC GTC GAA GAC ATC AGC AAG GAT GAT CCC GAG
 GAA TTC CAG TGC ACA CAA CAC CAT CTG TAG TCG TTC CTA CTA CGG CTC
 Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu>

 870 880 890 900 910
 GTC CAG TTC AGC TCG TTT GTC GAT GAT GTG GAG GTG CAC ACA GCT CAG
 CAG GTC AAG TCG ACC AAA CAT CTA CTA CAC CTC CAC GTG TGT CGA GTC
 Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln>

 920 930 940 950 960
 ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT
 TGC GTT GGG GCC CTC CTC GTC AAG TTG TCG TGA AAG GCG AGT CAG TCA
 Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser>

 970 980 990 1000
 GAA CTT CCC ATC ATG CAC CAG GAC TCG CTC AAT GGC AAG GAG TTC AAA
 CTT GAA GGG TAG TAC GTC CTC CTC ACC GAG TTA CCG TTC CTC AAC TTT
 Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys>

 1010 1020 1030 1040 1050
 * * * * *
 TGC AGG GTC AAC AGT GCA GCT TTC CCT CCC CCC ATC GAG AAA ACC ATC
 ACC TCC CAG TTG TCA CGT CGA AAG GCA CGG CGG TAG CTC TTT TCG TAG
 Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile>

 1060 1070 1080 1090 1100
 * * * * *
 TCC AAA ACC AAA GGC AGA CGG AAG GCT CCA CAG GTG TAC ACC ATT CCA
 AGG TTT TGG TTT CGG TCT GGC TTC CCA CCT GTC CAC ATG TCG TAA GGT
 Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro>

 1110 1120 1130 1140 1150
 * * * * *
 CCT CCC AAG GAG CAG ATG GCG AAG GAT AAA GTC AGT CTC ACC TGC ATG
 GGA CGG TTC CTC GTC TAC CGG TTC CTA TTT CAG TCA GAC TGG ACC TAC
 Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met>

 1160 1170 1180 1190 1200
 * * * * *
 ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTC GAG TGG CAG TGG AAT
 TAT TGT CTC AAG AAG GGA CTT CTC TAA TGA CAC CTC ACC GTC ACC TTA
 Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn>

 1210 1220 1230 1240
 * * * * *
 CGG CAG CCA CGG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA
 CCC GTC CGT CGC CTC TTC ATC TTC TTG TGA GTC GGG TAG TAC CTC TGT
 Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr>

 1250 1260 1270 1280 1290
 * * * * *
 GAT GGC TCT TAC TTC GTC TAC ACC AAG CTC AAT GTC CAG AAG ACC AAC
 CTA CGG AGA ATG AAG CAG ATC TCG TTC GAC TTA CAC GTC TTC TCG TTG
 Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn>

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FIG. 1 D

1300 1310 1320 1330 1340
TCG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG
ACC CTC CGT CCT TTA TGA AAG TCG ACC AGA CAC AAT GTA CTC CGG GAC
Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu>

1350 1360 1370 1380 1390
CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA TG ATC
CTG TTG GTG GTA TGA CTC TTC TCG GAG AGC GTG AGA GGA CCA TTT AC TAG
His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys>

1400 1410 1420 1430 1440
CCA GTG TCC TTG GAG CCC TCT GGT CCT ACA GGA CTC TGA CAC CTA CCT
GGT CAC AGG AAC CTC GGG AGA CCA GGA TGT CCT GAG ACT GTG GAT GGA

1450 1460 1470 1480
CCA CCC CTC CCT GTA TAA ATA AAG CAC CCA GCA CTC CCT TGG ACC C
GGT GGG GAG GGA CAT ATT TAT TTC GTG GGT GCG GAC GGA ACC TGG G

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Sequence of the murine TF8-5G9 light chain cDNA with protein translation. The essential regions of the cDNA are as follows:

FIG. 2 A

<u>Nucleotides</u>	<u>Region</u>
1-4	5' untranslated.
5-64	Start codon and leader sequence.
65-385	Variable region.
386-706	Murine kappa constant region.
707-917	3' untranslated region.
918-937	Poly A tail.

Sequence Range: 1 to 937

10	20	30	40	
GGA C ATG CGG CCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TCG TTT				
CCT G TAC GCC CGG CGA CGA GTC AAA AAA CCC TAG AAC AAC GAG ACC AAA				
Met Arg Ala Pro Ala Cln Phe Phe Gly Ile Leu Leu Leu Trp Phe>				
50	60	70	80	90
CCA CGT ATC AGA TGT GAC ATC AAC ATG ACC CAG TCT CCA TCC TCC ATG				
GGT CCA TAG TCT ACA CTG TAG TTC TAC TGG GTC AGA GGT AGG AGG TAC				
Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met>				
100	110	120	130	140
TAT CCA TCG CTG CGA GAG AGA GTC ACT ATC ACT TGT AAC CGG AGT CAG				
ATA CGT ACC GAC CCT CTC TCT CAG TGA TAG TGA ACA TTC CGC TCA GTC				
Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln>				
150	160	170	180	190
GAC ATT AGA AAC TAT TTA AAC TCG TAC CAG CAG AAA CCA TGG AAA TCT				
CTG TAA TCT TTC ATA AAT TTC ACC ATG GTC GTC TTT GGT ACC TTT AGA				
Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser>				
200	210	220	230	240
CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTC CCA GAT CGG GTC CCA				
CGA TTC TCG GAC TAC ATA ATA CGT TGT TCG AAC CGT CTA CCC CAG GGT				
Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro>				
250	260	270	280	
TCA AGA TTC ACT CGG ACT CGA TCT CGG CAA GAT TAT TCT CTA ACC ATC				
AGT TCT AAG TCA CGG TCA CCT AGA CCC GTT CTA ATA AGA GAT TGG TAG				
Ser Arg Phe Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile>				
290	300	310	320	330
AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT				
TCG TCG GAC CTC AGA CTG CTA TGT CGT TCA ATA ATG ACA GAT GTT GTC				
Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His>				

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FIG. 2B

340 350 360 370 380
 * * * * *
 GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC
 CCA CTC TCG GGC ATG TGC AAG CCT CCC CCC TGG TTC GAC CTT TAT TTG
 Gly Glu Ser Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile Asn>

 390 400 410 420 430
 * * * * *
 AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG
 TCC CCA CTA CGA CGT GGT TGA CAT AGG TAG AAG GGT GGT AGG TCA CTC
 Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu>

 440 450 460 470 480
 * * * * *
 CAC TTA ACA TCT GGA GGT CCC TCA GTC GTG TGC TTC TTG AAC AAC TTC
 GTC AAT TGT AGA CCT CCA CGG AGT CAG CAC ACG AAG AAC TTG TTG AAG
 Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe>

 490 500 510 520
 * * * *
 TAC CCC AAA GAC ATC ATC GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA
 ATG GGG TTT CTG TAG TTA CAG TTC ACC TTC TAA CTA CCC TCA CTT GCT
 Tyr Pro Lys Asp Ile Asn Val Lys Tyr Ile Asp Gly Ser Glu Arg>

 530 540 550 560 570
 * * * * *
 CAA AAT GGC GTC CTG AAC ACT TGG ACT GAT CAG GAC AGC AAA GAC AGC
 GTT TTA CGG CAG GAC TTG TCA ACC TGA CTA GTC CTG TCG TTT CTG TCG
 Gln Asn Gly Val Leu Asn Ser Tyr Thr Asp Gln Asp Ser Lys Asp Ser>

 580 590 600 610 620
 * * * * *
 ACC TAC ACC ATC ACC ACC CTC ACC TTC ACC AAG GAC GAG TAT GAA
 TCG ATG TCG TAC TCG TCG TGG GAG TGC AAC TGG TTC CTC CTC ATA CTT
 Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu>

 630 640 650 660 670
 * * * * *
 CGA CAT AAC ACC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA
 CCT GTC TTG TCG ATA TGG ACA CTC CGG TGA GTG TTC TGT AGT TGA AGT
 Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser>

 680 690 700 710 720
 * * * * *
 CCC ATT GTC AAG AGC TTC AAC AGG AAT GAC TGT TA GAG ACA AAG GTC CTC
 CGC TAA CAC TTC TCC AAG TTG TCC TTA CTC ACA AT CTC TGT TTC CAG GAC
 Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys>

 730 740 750 760 770
 * * * * *
 AGA CGC CAC CAC CAG CTC CCC ACC TCC ATC CTA TCT TCC CTT CTA AGG
 TCT CGC GTC GTG GTC GAG GGG TCG AGG TAG GAT AGA AGG GAA GAT TCC

 780 790 800 810
 * * * *
 TCT TGG AGG CTT CCC CAC AAG CGA CCT ACC ACT GTT GCG GTG CTC CAA
 AGA ACC TCC GAA GGG GTC TTC GCT GGA TGG TGA CAA CGC CAC GAG GTT

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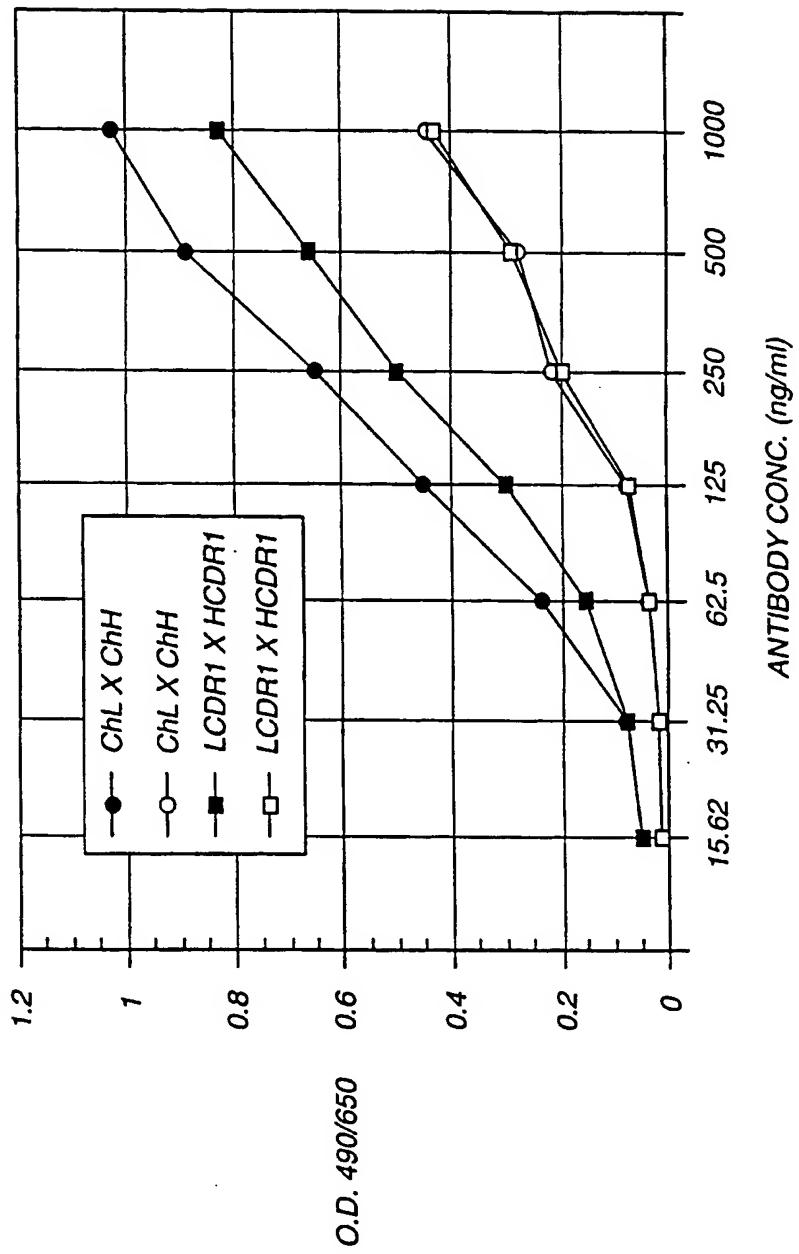
FIG. 2 C

820 830 840 850 860
* * * * *
ACC TCC TCC CCA CCT CCT TCT CCT CCT CCT CCC TTT CCT TGG CTT TTA
TGG AGG AGG GGT GGA GGA AGA GGA GGA GGA GGG AAA GGA ACC GAA AAT

870 880 890 900 910
* * * * *
TCA TGC TAA TAT TTG CAG AAA ATA TTC AAT AAA GTG AGT CTT TGC ACT
AGT ACG ATT ATA AAC GTC TTT TAT AAG TTA TTT CAC TCA GAA ACG TGA

920 930
* *
TGA AAA AAA AAA AAA AAA AAA AAA A
ACT TTT TTT TTT TTT TTT TTT T

FIG. 3
anti-TF BINDING AND COMPETITION ASSAYS



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FIG. 4 A

The pEe6TF8HCDR20 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted HC gene, TF8HCDR20, are translated.

Sequence Range: 1 to 7073

10	20	30	40	
GAA TTC GCC GCC ACC ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC TTG CTT AAG CGG CGG TGG TAC CTT ACC TCG ACC CAG AAA GAG AAG AAC AAC Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu>				
50	60	70	80	90
TCA GTA ACT ACA CGT GTA CAC TCA CAA GTT CAG CTG CTG GAG TCT GGA AGT CAT TGA TGT CCA CAT GTG AGT GTT CAA GTC GAC CAC CTC AGA CCT Ser Val Thr Thr Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly>				
100	110	120	130	140
GGA GGA GTA GTA CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT CCT CCT CAT CAT GTT GGA CCT TCC AGT GAC TCT GAC AGA ACA TTC CGA Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala>				
150	160	170	180	190
AGT GGA TTC AAT ATC AAG GAC TAT TAT ATG CAC TCG GTC AGA CAA CCT TCA CCT AAG TTA TAG TTC CTG ATA ATA TAC GTG ACC CAC TCT GTT CGA Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala>				
200	210	220	230	240
CCT CGA AAA CGA CTC GAC TCG ATA GGT TTA ATT GAT CCT GAG AAT CGT GGA CCT TTT CCT GAG CTC ACC TAT CCA AAT TAA CTA CGA CTC TTA CCA Pro Gly Lys Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly>				
250	260	270	280	
AAC ACC ATA TAT GAT CCC AAG TTC CAA CGA AGA TTC ACA ATT TCT CGA TTG TGC TAT ATA CTA CGG TTC AAG GTT CCT TCT AAG TGT TAA AGA CGT Asn Thr Ile Tyr Asp Pro Lys Phe Gln Gly Arg Phe Thr Ile Ser Ala>				
290	300	310	320	330
GAC AAC TCT AAC AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC ACA CCT CTG TTC AGA TTC TTA TCT GAC AAG GAC GTC TAC CTG AGT GAG TCT GGA Asp Asn Ser Lys Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro>				
340	350	360	370	380
GAG GAT ACA GCA GTC TAC TAT TGT CCT AGA GAT AAC AGT TAT TAC TTC CTC CTA TGT CGT CAC ATA ACA CGA TCT CTA TTG TCA ATA ATG AAG Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe>				
390	400	410	420	430
GAC TAC TGG CCC CAA CGA ACA CCA GTC ACC GTG ACC TCA GCT TCC ACC CTG ATG ACC CGG GTT CCT TGT GGT CAG TGG CAC TCG AGT CGA AGG TGG Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr>				

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FIG. 4 B

440 * 450 * 460 * 470 * 480 *
 AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC
 TTC CCG GGT AGG CAG AAG GGG GAC CGC GGG AGC AGG TCC TCG TGG AGG
 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser>

 490 * 500 * 510 * 520 *
 GAG AGC ACA GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA
 CTC TCG TGT CGG CGG GAC CCG AGC CAG TTC CTG ATG AAG GGG CTT
 Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu>

 530 * 540 * 550 * 560 * 570 *
 CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC
 GCC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG
 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His>

 580 * 590 * 600 * 610 * 620 *
 ACC TTC CCG GCT GTC CTA CAG TCC TCA CGA CTC TAC TCC CTC ACC ACC
 TGG AAG GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG
 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser>

 630 * 640 * 650 * 660 * 670 *
 GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC
 CAC CAC TGG CAC CGG AGG TCG TCG AAC CCG TGC TTC TGG ATG TGG ACC
 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys>

 680 * 690 * 700 * 710 * 720 *
 AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGT
 TTG CAT CTA GTG TTC GGG TCG TTG TGG TTC CAC CTG TTC TCT CAA CCA
 Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val>

 730 * 740 * 750 * 760 *
 GAG AGG CCA CCA CAG CGC AGG GAG GGT GTC TGC TGG AAG CCA CGC TCA
 CTC TCC GGT CGT GTC CGG TCC CTC CCA CAG AGC ACC TTC GGT CGG AGT

 770 * 780 * 790 * 800 * 810 *
 GCC CTC CTG CCT CGA CGC ACC CGG GGT GTG CAG CCC CAG CCC AGG GCA
 CGG GAG GAC CGA CCT CGG TCC CGG CCA CAC GTC CGG GTC CGG TCC CGT

 820 * 830 * 840 * 850 * 860 *
 GCA AGG CTT GCC CCA TCT GTC TCC TCA CCC CGA CGC CTC TGA CCA CCC
 CGT TCC GTA CGG GGT AGA CAG AGG AGT GGG CCT CGG GAG ACT GGT CGG

 870 * 880 * 890 * 900 * 910 *
 CAC TCA TGC TCA CGG AGA CGG TCT TCT CGA TTT TTC CAC CAG GCT CGG
 GTG AGT AGG AGT CCC TCT CCC AGA AGA CCT AAA AAG GTG GTC CGA CGC

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FIG. 4 C

920 930 940 950 960
 * * * * *
 CCC AGC CAC AGG CTC GAT GCC CCT ACC CCA GGC CCT GCG CAT ACA GGG
 CCC TCG GTC TCC GAC CTA CGG GGA TGG GGT CCC GGA CGC GTC TGT CCC

 970 980 990 1000
 * * * *
 GCA GGT GCT GCG CTC AGA CCT GCC AAG AGC CAT ATC CCG GAG GAC CCT
 CGT CCA CGA CGC GAG TCT GGA CGG TTC TCG GTC TAG GCC CTC CTG GGA

 1010 1020 1030 1040 1050
 * * * * *
 GCC CCT GAC CTA AGC CCA CCC CAA AGG CCA AAC TCT CCA CTC CCT CAG
 CGG GGA CTG GAT TCG GGT GGG GTT TCC GGT TTG AGA GGT GAC GGA GTC

 1060 1070 1080 1090 1100
 * * * * *
 CTC AGA CAC CTT CTC TCC CAG ATT CGA GTC ACT CCC AAT CTT CTC
 GAG TCT GTG GAA GAG AGG GTC TAA GCT CAT TCA CGG TTA GAA GAG

 1110 1120 1130 1140 1150
 * * * * *
 TCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA GGT AAG
 AGA CGT CTC AGG TTT ATA CCA GGG GGT AGC GGT AGT ACC GGT CCA TTC
 Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro>

 1160 1170 1180 1190 1200
 * * * * *
 CCA ACC CAG GCC TCG CCC TCC AGC TCA AGG CGG GAC AGG TCC CCT AGA
 GGT TGG GTC CGG AGC GGG AGG TCG AGT TCC CGG CTG TCC ACC GGA TCT

 1210 1220 1230 1240
 * * * *
 GTC CCC TCC ATC CAG CGA CAG GCC CCA CGC GGG TCC TGA CGC ATC CAC
 CAT CGG AGC TAG GTC CCT GTC CGG GGT CGG CCC AGC ACT CGC TAG GTG

 1250 1260 1270 1280 1290
 * * * * *
 CTC CAT CTC TTC CTC AGC A CCT GAC TTC CTC CGG CGA CCA TCA GTC TTC
 GAG GTC GAG AAG GAG TCC T GGA CTC AAG GAC CCC CCT GGT AGT CAG AAG
 Pro Glu Phe Leu Gly Pro Ser Val Phe>

 1300 1310 1320 1330 1340
 * * * * *
 CTC TTC CCC CCA AAA CCC AAG GAC ACT CTC ATC ATC TCC CGG ACC CCT
 GAC AAG CGG GGT TTT CGG TTC CTC TCA GAC TAC TAG AGG GCC TGG CGA
 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>

 1350 1360 1370 1380 1390
 * * * * *
 GAG GTC ACC TCC CTC GTG GTG GAC GTG ACC CAG GAA GAC CCC CGG GTC
 CTC CAG TCC ACC CAC CAC CTC CAC TCC GTC CTT CTG CGG CTC CAG
 Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val>

 1400 1410 1420 1430 1440
 * * * * *
 CAG TTC AAC TCG TAC GTG GAT GCC GTG GAG GTG CAT AAT GCC AAG ACA
 GTC AAG TTC ACC ATC CAC CTA CGG CAC CTC CAC GTC TTA CGG TTC TGT
 Gln Phe Asn Trip Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>

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FIG. 4 D

1450 1460 1470 1480
 * * * *
 AAG CCG CGG GAG GAC CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC
 TTC CGC CCC CTC CTC GTC AAG TTG TCG TGC ATG GCA CAC CAG TCG CAG
 Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val>

 1490 1500 1510 1520 1530
 * * * * *
 CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC
 GAG TGG CAG GAC GTG GTC CTG ACC GAC TTG CCG TTC CTC ATG TTC ACG
 Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>

 1540 1550 1560 1570 1580
 * * * * *
 AAG GTC TCC AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC
 TTC CAG AGG TTG TTT CCG GAG GGC AGG AGG TAG CTC TTT TGG TAG AGG
 Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser>

 1590 1600 1610 1620 1630
 * * * * *
 AAA GCC AAA GG TCG GAC CCA CGG CGT GCG AGG GCC ACA TGG ACA GAG GTC
 TTT CGG TTT CC ACC CTG GGT GCC CCA CCC TCC CGG TGT ACC TGT CTC CAG
 Lys Ala Lys>

 1640 1650 1660 1670 1680
 * * * * *
 AGC TCG GCC CAC CCT CTG CCC TGG GAG TGA CCG CTG TGC CAA CCT CTG
 TCG AGC CGG GTG GGA GAC GGG ACC CTC ACT GGC GAC ACG GTT GGA GAC

 1690 1700 1710 1720 1730
 * * * * *
 TCC CTA CA CGG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC
 AGG GAT GT CCC GTC CGG CCT CTC GGT GTC CAC ATG TGG GAC GGG GGT AGG
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser>

 1740 1750 1760 1770 1780
 * * * * *
 CAG GAG GAG ATG ACC AAG AAC CAG GTC ACC CTG ACC TGC CTG GTC AAA
 GTC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACC GAC CAG TTT
 Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys>

 1790 1800 1810 1820
 * * * * *
 CGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG
 CGG AAG ATG GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC
 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln>

 1830 1840 1850 1860 1870
 * * * * *
 CGC GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC
 CGC CTC TTC TTC ATC TTC TGG TGC GGA CGG CAC GAC CTC ACC CTG CCC
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly>

 1880 1890 1900 1910 1920
 * * * * *
 TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG
 AGG AAG AAG GAG ATG TCG TCC GAT TGG CAC CTC TTC TCG TCC ACC GTC
 Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln>

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FIG. 4 E

1930	1940	1950	1960	1970
GAG CCC AAT GTC TTC TCA TCC TCC GTG ATG CAT GAG GCT CTC CAC AAC				
CTC CCC TTA CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG				
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn>				
1980	1990	2000	2010	2020
CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT CTG GGT AAA T GAG TGC CAG				
GTG ATG TGT GTC TTC TCG GAG AGG GAC AGA GAC CCA TTT A CTC ACG GTC				
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Xox>				
2030	2040	2050	2060	2070
GCG CGG CAA GCC CCC GCT CCC CGG GCT CTC GGG GTC GCG CGA GGA TGC				
CGG CCC GTT CGG GGG CGA GGC GCG CGA GAG CCC CAG CGC GCT CCT ACG				
2080	2090	2100	2110	
TTC GCA CGT ACC CCG TCT ACA TAC TTC CCA GGC ACC CAG CAT CGA AAT				
AAC CGT GCA TGG GGC AGA TGT ATG AAG GGT CCG TGG GTC GTA CCT TTA				
2120	2130	2140	2150	2160
AAA GCA CCC ACC ACT GCC CTG GGC CCC TGT GAG ACT GTG ATG GTT CTT				
TTT CGT GGG TGG TGA CGG GAC CCC GGG ACA CTC TGA CAC TAC CAA GAA				
2170	2180	2190	2200	2210
TCC ACG GGT CAG GCC GAG TCT GAG GGC TGA GTG ACA TGA GGG AGG CAG				
AGG TCC CCA GTC CGG CTC AGA CTC CGG ACT CAC TGT ACT CCC TCC GTC				
2220	2230	2240	2250	2260
AGC GGG TCC CAC TGT CCC CAC ACT GGC CCA GGC TGT GCA GGT GTG CCT				
TGG CCC AGG GTG ACA GGG GTG TGA CGG GGT CGG ACA CGT CCA CAC GGA				
2270	2280	2290	2300	2310
GCG CCA CCT AGG GTC CGG CTC AGC CAG CGG CTG CCC TCG GCA GGG TGG				
CCC CGT GGA TCC CAC CCC GAC TCG CTC CCC GAC GGG AGC CGT CCC ACC				
2320	2330	2340	2350	
GCG ATT TGC CAG CGT GGC CCT CCC TCC AGC ACC AGG ACT CTA GAG GAT				
CCC TAA AGC GTC GCA CGG GGA GGG AGG TCG TCC TGA GAT CTC CTA				
2360	2370	2380	2390	2400
CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT TGC TTT AAA AAA				
CTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA ACC AAA TTT TTT				
2410	2420	2430	2440	2450
CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT GAA TCC AAT TGT				
GGA CGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA CTT ACC TTA ACA				

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FIG. 4 F

2460	2470	2480	2490	2500
TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA CAA ATA AAG CAA				
ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT GTT TAT TTC GTT				
2510	2520	2530	2540	2550
TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC ACT GCA TTC TAG				
ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG TGA CGT AAG ATC				
2560	2570	2580	2590	
TTC TCG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA TGT CTG GAT CCT				
AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT ACA GAC CTA GGA				
2600	2610	2620	2630	2640
CTA CGC CGG ACG CAT CGT GGC CGG CAT CAC CGG CGC CAC AGG TGC GGT				
GAT GCG GGC TGC GTA GCA CCG GCC GTA GTG GCC GCG GTG TCC ACC CCA				
2650	2660	2670	2680	2690
TGC TGG CGC CTA TAT CGC CGA CAT CAC CGA TGG GGA AGA TCG GGC TCG				
ACG ACC GCG GAT ATA GCG GCT GTA GTG GCT ACC CCT TCT AGC CGG ACC				
2700	2710	2720	2730	2740
CCA CTT CGG CCT CAT GAG CGC TTG TTT CGG CGT GGG TAT GGT GGC AGG				
GGT GAA GCC CGA GTA CTC CGG AAC AAA CCC GCA CCC ATA CCA CGG TCC				
2750	2760	2770	2780	2790
CCC GTG CCC CGG GGA CTG TTG GGC GCG ATC TCC TTG CRT GCA CCA TTC				
GGG CAC CGG CCC CCT GAC AAC CGG CGG TAG AGG AAC GTA CGT GGT AAG				
2800	2810	2820	2830	
CTT CGG CGC CGG GTG CTC AAC CGC CTC AAC CTA CTA CTG GGC TGC TTC				
GAA CGC CGC CGC CAC GAG TTG CGG GAG TTG GAT GAT GAC CGG AGC AAG				
2840	2850	2860	2870	2880
CTA ATG CAG GAG TCG CAT AAG GGA GAG CGT CGA CCT CGG CGC CGG TTG				
GAT TAC GTC CTC ACC GTA TTC CCT CTC GCA CCT GGA CGC CGC AAC				
2890	2900	2910	2920	2930
CTG CGG TTT TTC CAT AGG CTC CGC CGC CCT GAC GAG CAT CAC AAA AAT				
GAC CGC AAA AAG GTA TCC GAC CGG CGG GGA CTG CTC GTA GTG TTT TTA				
2940	2950	2960	2970	2980
CGA CGC TCA AGT CAG AGG TCG CGA AAC CGG ACA GGA CTA TAA AGA TAC				
GCT CGC AGT TCA GTC TCC ACC GCT TTG CGG TGT CCT GAT ATT TCT ATG				

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FIG. 4 G

2990 3000 3010 3020 3030
 CAC GCG TTT CCC CCT GGA AGC TCC CTC GTG CCC TCT CCT GTT CCG ACC
 GTC CGC AAA CGG GGA CCT TCG AGG GAG CAC CGG AGA CGA CAA CGC TGG

 3040 3050 3060 3070
 CTG CCG CTT ACC GGA TAC CTG TCC GCC TTT CTC CCT TCG GGA AGC GTG
 GAC CGC GAA TGG CCT ATG GAC AGG CGG AAA GAG GGA AGC CCT TCG CAC

 3080 3090 3100 3110 3120
 CGC CTT TCT CAA TCC TCA CGC TGT AGG TAT CTC AGT TCG GTG TAG GTC
 CGC GAA AGA GTT ACG AGT CGG ACA TCC ATA GAG TCA AGC CAC ATC CAG

 3130 3140 3150 3160 3170
 GTT CGC TCC AAG CTG GGC TGT GTG CAC GAA CCC CCC GTT CAG CCC GAC
 CAA CGG AGG TTC GAC CCG ACA CAC GTG CTT CGG GGG CAA GTC GGG CTG

 3180 3190 3200 3210 3220
 CGC TGC GCC TTA TCC CGT AAC TAT CGT CTT GAG TCC AAC CGG GTA AGA
 CGG ACG CGG AAT AGG CCA TTG ATA GCA GAA CTC AGG TTG CGC CAT TCT

 3230 3240 3250 3260 3270
 CAC GAC TTA TCG CCA CTG GCA GCA GCC ACT GGT AAC AGC AGG ATT AGC AGA
 GTG CTG AAT AGC GGT GAC CGT CGT CGG TGA CCA TTG TCC TAA TCG TCT

 3280 3290 3300 3310
 GCG AGG TAT GTA GGC GGT GCT ACA GAG TTC TTG AAG TCG TCG CCT AAC
 CCC TCC ATA CAT CGG CCA CGA TGT CTC AAG AAC TTC ACC ACC GGA TTG

 3320 3330 3340 3350 3360
 TAC CGC TAC ACT AGA AGG ACA GTA TTT GGT ATC TCC GCT CTC CTG AAG
 ATG CGG ATG TGA TCT TCC TGT CAT AAA CCA TAG AGC CGA GAC GAC TTC

 3370 3380 3390 3400 3410
 CCA GTT ACC TTC GGA AAA AGA GTT GGT AGC TCT TGA TCC CGC AAA CAA
 GGT CAA TCG AAG CCT TTT TCT CAA CCA TCG AGA ACT AGG CGG TTT GTT

 3420 3430 3440 3450 3460
 ACC ACC GCT GGT AGC GGT GGT TTT TTT GTT TCC AAG CAG CAG ATT AGC
 TGG TGG CGA CCA TCG CCA CCA AAA AAA CAA AGC TTC GTC GTC TAA TCC

 3470 3480 3490 3500 3510
 CGC AGA AAA AAA CGA TCT CAA GAA GAT CCT TTC ATC TTT TCT ACC CGG
 CGC TCT TTT TTT CCT AGA GTT CTT CTA CGA AAC TAG AAA AGA TGC CGG

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FIG. 4 H

3520 3530 3540 3550
 TCT GAC GCT CAG TCG AAC GAA AAC TCA CGT TAA GGG ATT TTG GTC ATG
 AGA CTG CGA GTC ACC TTC CTT TTG AGT GCA ATT CCC TAA AAC CAG TAC

 3560 3570 3580 3590 3600
 AGA TTA TCA AAA ACC ATC TTC ACC TAG ATC CTT TTA AAT TAA AAA TGA
 TCT AAT AGT TTT TCC TAG AAG TGG ATC TAG GAA AAT TTA ATT TTT ACT

 3610 3620 3630 3640 3650
 AGT TTT AAA TCA ATC TAA ACT ATA TAT GAG TAA ACT TGG TCT GAC AGT
 TCA AAA TTT AGT TAG ATT TCA TAT ATA CTC ATT TGA ACC AGA CTG TCA

 3660 3670 3680 3690 3700
 TAC CAA TGC TTA ATC AGT GAG GCA CCT ATC TCA CGG ATC TGT CTA TTT
 ATG GTT ACG AAT TAG TCA CTC CGT GGA TAG AGT CGC TAG ACA GAT AAA

 3710 3720 3730 3740 3750
 CGT TCA TCC ATA GTT GCC TGA CTC CCC GTC GTG TAG ATA ACT ACG ATA
 GCA AGT AGG TAT CAA CGG ACT GAG GGG CAG CAC ATC TAT TGA TGC TAT

 3760 3770 3780 3790
 CGG GAG GGC TTA CCA TCT GGC CCC AGT GCT GCA ATG ATA CGG CGA GAC
 GCC CTC CGG AAT GGT AGA CGG GGG TCA CGA CGT TAC TAT GGC GCT CTG

 3800 3810 3820 3830 3840
 CCA CGC TCA CGG GCT CCA GAT TTA TCA GCA ATA AAC CAG CCA GCC GGA
 GGT GCG AGT GGC CGA GGT CTA AAT AGT CGT TAT TTG GTC GGT CGG CCT

 3850 3860 3870 3880 3890
 AGG CCC GAG CGC AGA AGT GGT CCT GCA ACT TTA TCC GCC TCC ATC CAG
 TCC CGG CTC CGG TCT TCA CCA CGG CGT TGA AAT AGG CGG AGG TAG GTC

 3900 3910 3920 3930 3940
 TCT ATT AAT TGT TGC CGG GAA GCT AGA GTA AGT AGT TCG CCA GTT AAT
 AGA TAA TTA ACA ACC CGC CTT CGA TCT CAT TCA TCA AGC GGT CAA TTA

 3950 3960 3970 3980 3990
 AGT TTG CGC AAC GTT GTT GCC ATT GCT ACA CGC ATC GTC GTG TCA CGC
 TCA AAC CGC TTG CAA CAA CGG TAA CGA TGT CGG TAG CAC CAC AGT CGC

 4000 4010 4020 4030
 TCG TCG TTT CGT ATG GCT TCA TTC AGC TCC CGT TCC CAA CGA TCA AGG
 AGC AGC AAA CCA TAC CGA AGT AAG TCG AGG CGA AGC CGT GCT AGT TCC

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FIG. 4 I

4040 4050 4060 4070 4080
 CGA GTT ACA TCA TCC CCC ATG TTG TCC AAA AAA CCC GTT AGC TCC TTC
 CCT CAA TGT ACT ACC CCC TAC AAC ACG TTT TTT CCC CAA TCG AGG AAG

 4090 4100 4110 4120 4130
 GGT CCT CGG ATC CTT GTC AGA AGT AAG TTG GCC GCA GTG TTA TCA CTC
 CCA GGA CGC TAG CAA CAG TCT TCA TTC AAC CGG CGT CAC AAT AGT GAG

 4140 4150 4160 4170 4180
 ATG GTT ATG CCA CCA CTC CAT AAT TCT CTT ACT GTC ATG CCA TCC GTC
 TAC CAA TAC CCT CGT GAC CTA TTA AGA GAA TGA CAG TAC CGT AGG CTC

 4190 4200 4210 4220 4230
 AGA TGC TTT TCT GTG ACT GGT GAG TAC TCA ACC AAG TCA TTC TGA GAA
 TCT ACC AAA AGA CAC TGA CCA CTC ATG AGT TCG TTC AGT AAG ACT CTT

 4240 4250 4260 4270
 TAG TGT ATG CGG CGA CGG AGT TGC TCT TGC CGG CGG TCA ACA CGG GAT
 ATC ACA TAC GCC GCT CGC TCA ACC AGA ACC CGC CGC AGT TGT GCC CTC

 4280 4290 4300 4310 4320
 AAT ACC GCG CCA CAT AGC AGA ACT TTA AAA GTG CTC ATC ATT GGA AAA
 TTA TGG CGC CGT GTA TCG TCT TGA AAT TTT CAC GAG TAG TAA CCT TTT

 4330 4340 4350 4360 4370
 CGT TCT TCG CGG CGA AAA CTC TCA AGG ATC TTA CGG CTC TTG AGA TCC
 GCA AGA AGC CCC GCT TTT GAG AGT TCC TAG AAT CGC GAC AAC TCT AGG

 4380 4390 4400 4410 4420
 AGT TCG ATG TAA CCC ACT CGT GCA CCC AAC TGA TCT TCA GCA TCT TTT
 TCA AGC TAC ATT CGG TGA GCA CGT CGG TTG ACT AGA AGT CGT AGA AAA

 4430 4440 4450 4460 4470
 ACT TTC ACC AGC GTT TCT CGG TGA GCA AAA ACA GGA AGG CAA AAT CGC
 TGA AAG TGG TCG CAA AGA CCC ACT CGT TTT TGT CCT TCC GTT TTA CGG

 4480 4490 4500 4510
 GCA AAA AAG CGA ATA AGG CGG ACA CGG AAA TGT TGA ATA CTC ATA CTC
 CGT TTT TTC CCT TAT TCC CGC TGT GCC TTT ACA ACT TAT GAG TAT GAG

 4520 4530 4540 4550 4560
 TTC CTT TTT CAA TAT TAT TGA AGC ATT TAT CAG CGT TAT TGT CTC ATG
 AAG GAA AAA GTT ATA ATA ACT TCG TAA ATA GTC CCA ATA ACA GAG TAC

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FIG. 4 J

4570 4580 4590 4600 4610
 * * * * *
 AGC CGA TAC ATA TTT GAA TGT ATT TAG AAA AAT AAA CAA ATA CGG GTT
 TCG CCT ATG TAT AAA CTT ACA TAA ATC TTT TTA TTT GTT TAT CCC CAA

 4620 4630 4640 4650 4660
 * * * * *
 CGC CGG ACA TTT CCC CGA AAA GTG CCA CCT GAC GTC TAA GAA ACC ATT
 CCC CGG TGT AAA GGG GCT TTT CAC CCT CGA CTG CAG ATT CTT TGG TAA

 4670 4680 4690 4700 4710
 * * * * *
 ATT ATC ATG ACA TTA ACC TAT AAA AAT AGG CGT ATC ACC AGG CCC TGA
 TAA TAG TAC TGT AAT TCG ATA TTT TTA TCC GCA TAG TGC TCC GGG ACT

 4720 4730 4740 4750
 * * * *
 TGG CTC TTT CGG CGA CCC ATC GTT CGT AAT GTT CGG TGG CAC CGA CGA
 ACC GAG AAA CGC CGT CGG TAG CAA GCA TTA CAA CGC ACC GTC CCT CCT

 4760 4770 4780 4790 4800
 * * * * *
 CAA CCC TCA AGA GAA AAT CTA ATC ACA CTG CCT CAC CTT CGG GTG CGC
 GTT CGG AGT TCT CTT TTA CAT TAG TGT GAC CGA GTC GAA CGC CAC CCG

 4810 4820 4830 4840 4850
 * * * * *
 CTT TCT CGG TTT ATA AGG AGA CAC TTT ATG TTT AAG AAG GTT GGT AAA
 GAA AGA CGC AAA TAT TCC TCT GTG AAA TAC AAA TTC TTC CAA CGA TTT

 4860 4870 4880 4890 4900
 * * * * *
 TTC CTT CGG GCT TTG CGA CGC AAG CTA GAG ATC TCT AGC TTC GTG TCA
 AAG GAA CGC CGA AAC CGT CGG TTC GAT CTC TAG AGA TCG AAG CAC AGT

 4910 4920 4930 4940 4950
 * * * * *
 AGG ACG GTG ACT CGA GTG AAT AAT AAA ATG TGT GTT TGT CCG AAA TAC
 TCC TGC CAC TGA CGT CAC TTA TTA TTT TAC ACA CAA ACA CGC TTT ATG

 4960 4970 4980 4990
 * * * *
 CGC TTT TGA GAT TTC TGT CGC CGA CTA AAT TCA TGT CGC CGG ATA GTG
 CGC AAA ACT CTA AAG ACA CGG GCT GAT TTA AGT ACA CGC CGC TAT CAC

 5000 5010 5020 5030 5040
 * * * * *
 GTG TTT ATC CGC GAT AGA GAT CGC GAT ATT CGA AAA ATC GAT ATT TGA
 CAC AAA TAG CGG CTA TCT CTA CGG CTA TAA CCT TTT TAG CTA TAA ACT

 5050 5060 5070 5080 5090
 * * * * *
 AAA TAT CGC ATA TTG AAA ATG TCG CGG ATG TGA GTT TCT GTG TAA CTG
 TTT ATA CGG TAT AAC TTT TAC AGC CGC TAC ACT CAA AGA CAC ATT GAC

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FIG. 4 K

5100	5110	5120	5130	5140
ATA TCG CCA TTT TTC CAA AAG TGA TTT TTG GGC ATA CGC GAT ATC TGG TAT AGC GGT AAA AAG GTT TTC ACT AAA AAC CGG TAT CGG CTA TAG ACC				
5150	5160	5170	5180	5190
CGA TAG CGC TTA TAT CGT TTA CGG CGG ATG GGC ATA GAC GAC TTT GGT GCT ATC CGG AAT ATA CGA AAT CGC CCC TAC CGC TAT CTG CTG AAA CGA				
5200	5210	5220	5230	
GAC TTG CGC GAT TCT GTG TGT CCC AAA TAT CGC AGT TTC GAT ATA CGT CTG AAC CGG CTA AGA CAC ACA CGG TTT ATA CGG TCA AAG CTA TAT CGA				
5240	5250	5260	5270	5280
GAC AGA CGA TAT GAG GCT ATA TCG CGC ATA GAG CGG ACA TCA ACC TGG CTG TCT GCT ATA CTC CGA TAT ACC CGC TAT CTC CGC TGT ACT TCG ACC				
5290	5300	5310	5320	5330
CAC ATG GCC AAT CGA TAT CGA TCT ATA CAT TGA ATC AAT ATT CGC CAT GTG TAC CGG TTA CGT ATA CGT AGA TAT GTA ACT TAG TTA TAA CGG GTA				
5340	5350	5360	5370	5380
TAG CGA TAT TAT TCA TTG GTT ATA TAG CAT AAA TCA ATA TTG GCT ATT ATC CGT ATA ATA ACT AAC CAA TAT ATC GTA TTT AGT TAT AAC CGA TAA				
5390	5400	5410	5420	5430
GGC CAT TGC ATA CGT TGT ATC CAT ATC ATA ATA TGT ACA TTT ATA TTG CGG GTA ACG TAT CGA ACA TAG GTA TAG TAT ACA TGT AAA TAT AAC				
5440	5450	5460	5470	
GCT CAT GTC CAA CAT TAC CGC CAT GTT GAC ATT GAT TAT TGA CTA GTT CGA GTA CAG GTT GTA ATG CGG GTA CAA CTG TAA CTA ATA ACT GAT CAA				
5480	5490	5500	5510	5520
ATT AAT AGT AAT CAA TTA CGG GGT CAT TAG TTC ATA GCC CAT ATA TGG TAA TTA TCA TTA GTT AAT CGC CGA GTA ATC AAG TAT CGG GTA TAT ACC				
5530	5540	5550	5560	5570
AGT TCC CGG TTA CAT AAC TTA CGG TAA ATG GCC CGC CGC CTG GCT GAC CGC TCA AGG CGC AAT GTA TTG AAT GCC ATT TAC CGG CGG GAC CGA CTG CGG				
5580	5590	5600	5610	5620
CCA ACG ACC CCC CGC CAT TGA CGT CAA TAA TGA CGT ATG TTC CCA TAG GGT TGC TGG GGG CGG GTA ACT GCA GTT ATT ACT GCA TAC AAG GGT ATC				

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FIG. 4 L

5630 5640 5650 5660 5670
 * * * * *
 TAA CGC CAA TAG GGA CTT TCC ATT GAC GTC AAT GGG TGG AGT ATT TAC
 ATT GCG GTT ATC CCT GAA AGG TAA CTG CAG TTA CCC ACC TCA TAA ATG

 5680 5690 5700 5710 5720
 * * * * *
 GGT AAA CTG CCC ACT TCG CAG TAC ATC AAG TGT ATC ATA TGC CAA GTA
 CCA TTT GAC GGG TGA ACC GTC ATG TAG TTC ACA TAG TAT ACC GTT CAT

 5720 5730 5740 5750 5760
 * * * * *
 CCC CCC CTA TTG ACG TCA ATG ACG GTA AAT CCC CGG CCT CCC ATT ATG
 CCC GGG GAT AAC TGC AGT TAC TGC CAT TTA CCC GGC GGA CCC TAA TAC

 5770 5780 5790 5800 5810
 * * * * *
 CCC ACT ACA TGA CCT TAT GGG ACT TTC CTA CTT GGC AGT ACA TCT ACC
 GGG TCA TGT ACT GGA ATA CCC TGA AAG GAT GAA CCG TCA TGT AGA TGC

 5820 5830 5840 5850 5860
 * * * * *
 TAT TAG TCA TCG CTA TTA CCA TGG TGA TGC GGT TTT GGC AGT ACA TCA
 ATA ATC AGT ACC GAT AAT GGT ACC ACT ACC CCA AAA CCG TCA TGT AGT

 5870 5880 5890 5900 5910
 * * * * *
 ATG GGC GTG GAT ACC GGT TTG ACT CAC CGG GAT TTC CAA GTC TCC ACC
 TAC CCC CAC CTA TCG CCA AAC TGA GTG CCC CTA AAG GTT CAG ACC TGG

 5920 5930 5940 5950 5960
 * * * * *
 CCA TTG ACG TCA ATG GGA GTT TGT TTT GGC ACC AAA ATC AAC GGG ACT
 GGT AAC TGC AGT TAC CCT CAA ACA AAA CCG TGG TTT TAG TTG CCC TGA

 5960 5970 5980 5990 6000
 * * * * *
 TTC CAA AAT GTC GTA ACA ACT CCC CCC CAT TGA CGC AAA TGG CCC GTC
 AAG GTT TTA CAG CAT TGT TGA CGC GGG GTA ACT CGC TTT ACC CCC CAT

 6010 6020 6030 6040 6050
 * * * * *
 GGC GTG TAC GGT GGG AGG TCT ATA TAA GCA GAG CTC GTT TAG TGA ACC
 CCG CAC ATG CCA CCC TCC AGA TAT ATT CGT CTC GAG CAA ATC ACT TGG

 6060 6070 6080 6090 6100
 * * * * *
 GTC AGA TCG CCT GGA GAC GCC ATC CAC GCT GTT TTG ACC TCC ATA GAA
 CAG TCT AGC GGA CCT CTG CGG TAG GTG CGA CAA AAC TGG AGG TAT CTT

 6110 6120 6130 6140 6150
 * * * * *
 GAC ACC GGG ACC GAT CCA GGC TCC GCG GCC GGG AAC CGT CGA TTG GAA
 CTG TGG CCC TGG CTA GGT CGG AGG CGC CGG CCC TTG CCA CGT AAC CTT

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FIG. 4 M

6160	6170	6180	6190	
CGC GGA TTC CCC GTG CCA AGA GTG ACG TAA GTA CGG CCT ATA GAG TCT				
CGC CCT AAG GGG CAC CGT TCT CAC TGC ATT CTT CCC GGA TAT CTC AGA				
6200	6210	6220	6230	6240
ATA CCC CCA CCC CCT TGG CTT CTT ATG CAT GCT ATA CTG TTT TTG GCT				
TAT CGG CGT CCC GGA ACC GAA TAC GTA CGA TAT GAC AAA AAC CGA				
6250	6260	6270	6280	6290
TGG CGT CTA TAC ACC CCC GCT TCC TCA TGT TAT AGG TGA TGG TAT AGC				
ACC CCA GAT ATG TGG CGG CGA AGG AGT ACA ATA TCC ACT ACC ATA TCG				
6300	6310	6320	6330	6340
TTA GCC TAT AGG TGT CGG TTA TTG ACC ATT ATT GAC CAC TCC CCT ATT				
AAT CGG ATA TCC ACA CCC AAT AAC TGG TAA TAA CTG GTG AGG CGA TAA				
6350	6360	6370	6380	6390
GGT GAC GAT ACT TTC CAT TAC TAA TCC ATA ACA TGG CTC TTT CCC ACA				
CCA CTG CTA TGA AAC GTA ATG ATT AGG TAT TGT ACC GAG AAA CGG TGT				
6400	6410	6420	6430	
ACT CTC TTT ATT CGC TAT ATG CCA ATA CAC TGT CCT TCA GAG ACT GAC				
TGA GAG AAA TAA CGG ATA TAC CGT TAT GTG ACA CGA ACT CTC TGA CTG				
6440	6450	6460	6470	6480
ACG GAC TCT GTA TTT TTA CAG GAT CGG GTC TCA TTT ATT ATT TAC AAA				
TGC CTG AGA CAT AAA AAT GTC CTA CCC CAG AGT AAA TAA TAA ATG TTT				
6490	6500	6510	6520	6530
TTC ACA TAT ACA ACA CCA CGG TCC CCA GTG CCC GCA GTT TTT ATT AAA				
AAG TGT ATA TGT TGT GGT CGC AGG CGT CAC CGG CGT CAA AAA TAA TTT				
6540	6550	6560	6570	6580
CAT AAC GTG CGA TCT CCA CGG GAA TCT CGG GTA CGT GTT CGG GAC ATG				
GTA TTG CAC CCT AGA CGT CGC CTT AGA GCC CAT CGA CAA CGC CTG TAC				
6590	6600	6610	6620	6630
GGC TCT TCT CGG GTA CGG CGG GAG CTT CTA CAT CGG AGC CCT GCT CCC				
CGG AGA AGA CGC CAT CGC CGG CTC GAA GAT GTA CGC TCG CGA CGA CGG				
6640	6650	6660	6670	
ATG CCT CCA CGG ACT CAT GGT CGC TCG CGA GCT CCT TGC TCC TAA CAG				
TAC CGA CGT CGC TGA GTA CGA CGG AGC CGT CGA CGA AGC AGG ATT GTC				

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FIG. 4 N

6680 6690 6700 6710 6720
 * * * * *
 TGG AGG CCA GAC TTA GGC ACA GCA CGA TGC CCA CCA CCA CCA GTC TGC
 ACC TCC GGT CTG AAT CCC TGT CGT GCT ACG GGT GGT GGT GGT CAC ACG

 6730 6740 6750 6760 6770
 * * * * *
 CGC ACA AGG CGG TGG CGG TAG GGT ATG TGT CTG AAA ATG AGC TCG GGG
 CGG TGT TCC CGC ACC GCC ATC CCA TAC ACA GAC TTT TAC TCG AGC CCC

 6780 6790 6800 6810 6820
 * * * * *
 AGC GGG CTT GCA CGG CTG ACG CAT TTG GAA GAC TTA AGG CAG CGG CAG
 TCG CCC GAA CGT CGC GAC TGC GTA AAC CTT CTG AAT TCC GTC GCC GTC

 6830 6840 6850 6860 6870
 * * * * *
 AAC AAG ATG CAG GCA GCT GAG TTG TTC TGT TCT GAT AAG ACT CAG AGG
 TTC TTC TAC GTC CGT CGA CTC AAC AAC ACA AGA CTA TTC TCA GTC TCC

 6880 6890 6900 6910
 * * * *
 TAA CTC CGG TTG CGG TGC TGT TAA CGG TGG AGG GCA GTC TAG TCT GAG
 ATT GAG CGC AAC GCC ACC ACA ATT GCC ACC TCC CGT CAC ATC AGA CTC

 6920 6930 6940 6950 6960
 * * * * *
 CAG TAC TCC TTG CTG CGG CGC CGG CCA CCA GAC ATA ATA GCT GAC AGA
 GTC ATG ACC AAC GAC CGC CGG CGC CGT CGT CTG TAT TAT CGA CTG TCT

 6970 6980 6990 7000 7010
 * * * * *
 CTA ACA GAC TGT TCC TTT CCA TGG GTC TTT TCT GCA GTC ACC GTC CTT
 GAT TGT CTG ACA AGG AAA CGT ACC CAG AAA AGA CGT CAG TGG CAG GAA

 7020 7030 7040 7050 7060
 * * * * *
 GAC ACG AAG CTT CGG CTG CAG GTC GAT CGA CTC TAG AGG ATC GAT CCC
 CTG TGC TTC GAA CCC GAC GTC CAG CTA GCT GAG ATC TCC TAG CTA GGG

 7070
 *
 CGG CGG AGC TC
 CGC CGC TCC AG

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FIG. 5 A

The pEe12TF8LCDR3 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted LC gene, TF8LCDR3, are translated.

Sequence Range: 1 to 7864

10	20	30	40	50
*	*	*	*	*
AAT TCA CC ATG GGT GTG CCA ACT CAG GTA TTA GGA TTA CTG CTG CTG TGG TTA AGT GG TAC CCA CAC GGT TGA GTC CAT AAT CCT AAT GAC GAC GAC ACC Met Gly Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp>				
60	70	80	90	
*	*	*	*	
CTT ACA GAT GCA AGA TCT GAT ATC CAA ATG ACA CAA TCT CCT TCT TCT GAA TGT CTA CGT TCT ACA CTA TAG GTT TAC TGT GTT AGA GGA AGA AGA Leu Thr Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser>				
100	110	120	130	140
*	*	*	*	*
CTA AGT GCT TCT GTC GGA GAT AGA GTA ACA ATT ACA TGT AAG GGG AGT GAT TCA CGA AGA CAG CCT CTA TCT CTT TGT TAA TGT ACA TTC CGC TCA Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser>				
150	160	170	180	190
*	*	*	*	*
CAG GAC ATT AGA AAG TAT TTA AAC TGG TAT CAG CAA AAA CCT GGG AAG GTC CTG TAA TCT TTC ATA AAT TTG ACC ATA GTC GTT TTT GGA CCC TTC Gln Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys>				
200	210	220	230	240
*	*	*	*	*
GCT CCT AAG CTA CTG ATT TAT TAT GCA ACA AGT TTC GCA GAT GGA GTA CGA GGA TTC GAT GAC TAA ATA ATA CGT TGT TCA AAC CGT CTA CCT CAT Ala Pro Lys Leu Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val>				
250	260	270	280	290
*	*	*	*	*
CCT TCT AGA TTT TCT CGT TCT CCC TCT CGA ACA GAC TAC ACA TTC ACA GGA AGA TCT AAA AGA CCA AGA CGG AGA CCT TGT CGT ATG TGT AAG TGT Pro Ser Arg Phe Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr>				
300	310	320	330	
*	*	*	*	
ATT TCT TCT CTC CAA CCT GAG GAC ATT CCT ACA TAC TAC TGC CTA CAA TAA AGA AGA GAG GTT CGA CTC CTG TAA CGA TGT ATG ATG ACC GAT GTT Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln>				
340	350	360	370	380
*	*	*	*	*
CAT GGT GAG ACT CGG TAT ACA TTT GGA CAA CGA ACA AAA CTA GAG ATC GTA CGA CTC TCA CGC ATA TGT AAA CCT GTT CCT TGT TTT GAT CTC TAC His Gly Glu Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile>				
390	400	410	420	430
*	*	*	*	*
ACA AGA ACT GTT CGG CGC CGG TCT GTC TTC ATC TTC CGG CCA TCT GAT TGT TCT TGA CAA CGC CGC AGA CAG AAC TAG AAC GGC GGT AGA CTA Thr Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp>				

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FIG. 5 B

440 450 460 470 480
 * * * * *
 GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC
 CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG
 Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn>

 490 500 510 520 530
 * * * * *
 TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC CCC CTC
 AAG ATA GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG
 Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu>

 540 550 560 570
 * * * *
 CAA TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC
 GTT AGC CCA TTG AGG GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG
 Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp>

 580 590 600 610 620
 * * * * *
 AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC
 TCG TGG ATG TCG GAG TCG TGG GAC TGC GAC TCG TTT CGT CTG ATG
 Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr>

 630 640 650 660 670
 * * * * *
 GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
 CTC TTT GTG TTT CAG ATG CCG ACG CTT CAG TCG GTA GTC CCG GAC TCG
 Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser>

 680 690 700 710 720
 * * * * *
 TCG CCC GTC ACA AAG AGC TTC AAC AGC GGA GAG TGT T AGA GGG AGA AGT
 AGC GGG CAG TGT TTC TCG AAG TTG TCC CCT CTC ACA A TCT CCC TCT TCA
 Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys>

 730 740 750 760 770
 * * * * *
 CCC CCC ACC TGC TCC TCA GTT CCA GCC TGG GGA TCA TAA TCA CCC ATA
 CCC GGG TGG ACG AGG AGT CAA GGT CGG ACC CCT AGT ATT ACT CGG TAT

 780 790 800 810
 * * * * *
 CCA CAT TTG TAG AGC TTT TAC TTG CTT TAA AAA ACC TCC CAC ACC TCC
 GGT GTA AAC ATC TCC AAA ATC AAC GAA ATT TTT TGG ACC GTC TGG AGG

 820 830 840 850 860
 * * * * *
 CCC TGA ACC TGA AAC ATA AAA TGA ATG CAA TTG TTG TTG TTA ACT TGT
 GGG ACT TGG ACT TTG TAT TTT ACT TAC GTT AAC AAC AAT TGA ACA

 870 880 890 900 910
 * * * * *
 TTA TTG CAG CTT ATA ATG GTT ACA AAT AAA GCA ATA GCA TCA CAA ATT
 AAT AAC GTC GAA TAT TAC CAA TGT TTA TTT CGT TAT CGT AGT GTT TAA

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FIG. 5 C

920 930 940 950 960
 * * * * *
 TCA CAA ATA AAG CAT TTT TTT CAC TGC ATT CTA GTT GTG GTT TGT CCA
 AGT GTT TAT TTC GTC AAA AAA GTG ACG TAA GAT CAA CAC CAA ACG GGT

 970 980 990 1000 1010
 * * * * *
 AAC TCA TCA ATG TAT CTT ATC ATG TCT GGA TCC TCT ACG CCG GAC GCA
 TTG AGT AGT TAC ATA GAA TAG TAC AGA CCT AGG AGA TGC GGC CTG CGT

 1020 1030 1040 1050
 * * * *
 TCG TGG CCG GCA TCA CCG GGG CCA CAG GTG CGG TTG CTG GCG CCT ATA
 AGC ACC GGC CGT AGT GGC CGC CGT GTC CAC CCC AAC GAC CGC GGA TAT

 1060 1070 1080 1090 1100
 * * * * *
 TCG CCG ACA TCA CCG ATG GGG AAG ATC GGG CTC CCC ACT TCG GGC TCA
 AGC GGC TGT AGT GGC TAC CCC TTC TAG CCC GAG CGG TGA AGC CGC AGT

 1110 1120 1130 1140 1150
 * * * * *
 TGA GCG CTT GTT TCG GCG TGG GTA TGG TGG CAG GCG CGT GGC CGG CGG
 ACT CGC GAA CAA AGC CGC ACC CAT ACC ACC GTC CGG GCA CGG CCC CCC

 1160 1170 1180 1190 1200
 * * * * *
 ACT GTT GGG CGC CAT CTC CTT GCA TGC ACC ATT CCT TGC GGC GGC CGT
 TGA CAA CCC CGG GTC GAG GAA CGT AGC TGG TAA GGA AGC CGG CGG CCA

 1210 1220 1230 1240 1250
 * * * * *
 GCT CAA CGG CCT CAA CCT ACT ACT GGG CTC CTT CCT AAT GCA GGA GTC
 CGA GTT GCC GGA GTT GGA TGA TGA CCC GAC GAA GGA TTA CGT CCT CAG

 1260 1270 1280 1290
 * * * *
 GCA TAA CGG AGA GCG TCG ACC TCG GGC CGC GTT GCT GGC GTT TTT CCA
 CGT ATT CCC TCT CGC AGC TGG AGC CGG CGG CAA CGA CGG CAA AAA CGT

 1300 1310 1320 1330 1340
 * * * * *
 TAG GCT CGG CCC CCC TGA CGA GCA TCA CAA AAA TCG ACG CTC AAG TCA
 ATC CGA CGC GGG GGG ACT GCT CCT AGT GTT TTT AGC TGC GAC TTC AGT

 1350 1360 1370 1380 1390
 * * * * *
 GAG GTG GCG AAA CCC GAC AGG ACT ATA AAG ATA CCA GGC GTT TCC CCC
 CTC CAC CGC TTT CGG CTC TCC TGA TAT TTC TAT CGT CGG CAA AGG GGG

 1400 1410 1420 1430 1440
 * * * * *
 TCG AAG CTC CCT CGT CGG CTC TCC TGT TCC GAC CCT GCC CCT TAC CGG
 ACC TTC GAC CGA CGA CGC GAC AGG AGA CGC CGT CGA CGG CGA ATG CGC

 1450 1460 1470 1480 1490
 * * * * *
 ATA CCT GTC CGC CTT TCT CCC TTC CGG AAG CGT CGC CCT TTC TCA ATG
 TAT CGA CAG CGC GAA AGA CGG AAG CCC TTC GCA CGG CGA AAG AGT TAC

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FIG. 5 D

1500 1510 1520 1530
 * * * *
 CTC ACG CTG TAG GTA TCT CAG TTC GGT GTA GGT CGT TCG CTC CAA GCT
 GAG TGC GAC ATC CAT AGA GTC AAG CCA CAT CCA GCA AGC GAG GTT CGA

 1540 1550 1560 1570 1580
 * * * * *
 GGG CTG TGT GCA CGA ACC CCC CGT TCA GCC CGA CGG CTG CGC CTT ATC
 CCC GAC ACA CGT GCT TGG GGG GCA AGT CGG GCT GGC GAC GCG GAA TAG

 1590 1600 1610 1620 1630
 * * * * *
 CGG TAA CTA TCG TCT TGA GTC CAA CCC GGT AAG ACA CGA CTT ATC GCC
 GCC ATT GAT AGC AGA ACT CAG GTT GGG CCA TTC TGT GCT GAA TAG CGG

 1640 1650 1660 1670 1680
 * * * * *
 ACT GGC AGC AGC CAC TGG TAA CAG GAT TAG CAG AGC GAG GTA TGT AGG
 TGA CGG TCG TCG GTG ACC ATT GTC CTA ATC GTC TCG CTC CAT ACA TCC

 1690 1700 1710 1720 1730
 * * * * *
 CGG TGC TAC AGA GTT CTT GAA GTG GTG GCC TAA CTA CGG CTA CAC TAG
 GCC ACG ATG TCT CAA GAA CTT CAC CAC CGG ATT GAT GCC GAT GTG ATC

 1740 1750 1760 1770
 * * * *
 AAG GAC AGT ATT TGG TAT CTG CGC TCT GCT GAA GCC AGT TAC CTT CGG
 TTC CTG TCA TAA ACC ATA GAC CGG AGA CGA CTT CGG TCA ATG GAA GCC

 1780 1790 1800 1810 1820
 * * * * *
 AAA AAG AGT TGG TAG CTC TTG ATC CGG CAA ACA AAC CAC CGG TGG TAG
 TTT TTC TCA ACC ATC GAG AAC TAG GCC GTT TGT TTG GTG GCG ACC ATC

 1830 1840 1850 1860 1870
 * * * * *
 CGG TCG TTT TTT TGT TTG CAA GCA GCA GAT TAC CGG CAG AAA AAA AGG
 GCC ACC AAA AAA ACA AAC GTT CGT CGT CTA ATG CGC GTC TTT TTT TCC

 1880 1890 1900 1910 1920
 * * * * *
 ATC TCA AGA AGA TCC TTT GAT CTT TTC TAC CGG GTC TGA CGC TCA GTG
 TAG AGT TCT TCT AGC AAA CTA GAA AAG ATG CCC CAG ACT CGG AGT CAC

 1930 1940 1950 1960 1970
 * * * * *
 GAA CGA AAA CTC ACC TTA AGG GAT TTT GGT CAT GAG ATT ATC AAA AAG
 CTT GCT TTT GAG TGC AAT TCC CTA AAA CCA GTC TAA TAG TTT TTC

 1980 1990 2000 2010
 * * * *
 GAT CTT CAC CTA GAT CCT TTT AAA TTA AAA ATG AAG TTT TAA ATC AAT
 CTA GAA GTG GAT CTA GGA AAA TTT AAT TTT TAC TTC AAA ATT TAG TTA

 2020 2030 2040 2050 2060
 * * * * *
 CTA AAG TAT ATA TGA GTC AAC TTG GTC TGA CAG TTA CCA ATG CTT AAT
 GAT TTC ATA TAT ACT CAT TTG AAC CAG ACT GTC AAT GGT TAC GAA TTA

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FIG. 5 E

2070	2080	2090	2100	2110
*	*	*	*	*
CAG TGA GGC ACC TAT CTC AGC GAT CTG TCT ATT TCG TTC ATC CAT AGT				
GTC ACT CCG TCG ATA GAG TCG CTA GAC AGA TAA AGC AAG TAG GTA TCA				
2120	2130	2140	2150	2160
*	*	*	*	*
TGC CTG ACT CCC CGT CGT GTA GAT AAC TAC GAT ACG GGA GGG CTT ACC				
ACG GAC TGA GGG GCA GCA CAT CTA TTG ATG CTA TGC CCT CCC GAA TGG				
2170	2180	2190	2200	2210
*	*	*	*	*
ATC TGG CCC CAG TGC TGC AAT GAT ACC GCG AGA CCC ACG CTC ACC GGC				
TAG ACC GGG GTC ACG ACG TTA CTA TGG CGC TCT GGG TGC GAG TGG CCC				
2220	2230	2240	2250	
*	*	*	*	
TCC AGA TTT ATC AGC AAT AAA CCA GCC AGC CGG AAG GGC CGA GCG CAG				
AGG TCT AAA TAG TCG TTA TTT GGT CGG TCG GCC TTC CCC GCT CCC GTC				
2260	2270	2280	2290	2300
*	*	*	*	*
AAC TGG TCC TGC AAC TTT ATC CGC CTC CAT CCA GTC TAT TAA TTG TTG				
TTC ACC ACC ACC TTG AAA TAG CGG GAG GTA GGT CAG ATA ATT AAC AAC				
2310	2320	2330	2340	2350
*	*	*	*	*
CCG GGA AGC TAG AGT AAG TAG TTC GCC AGT TAA TAG TTT GCG CAA CGT				
GCC CCT TCG ATC TCA TTC ATC AAG CGG TCA ATT ATC AAA CCC GTT GCA				
2360	2370	2380	2390	2400
*	*	*	*	*
TGT TGC CAT TGC TAC AGG CAT CGT GGT GTC ACG CTC GTC GTT TGG TAT				
ACA ACC GTA ACC ATG TCC GTA CCA CCA CAG TGC GAG CAG CAA ACC ATA				
2410	2420	2430	2440	2450
*	*	*	*	*
GCC TTC ATT CAG CTC CGG TTC CCA ACC ATC AAG GCG AGT TAC ATG ATC				
CCC AAG TAA GTC GAG GCC AAG GGT TCC TAG TTC CGC TCA ATC TAC TAG				
2460	2470	2480	2490	
*	*	*	*	
CCC CAT GTT GTG CAA AAA AGC GGT TAG CTC CTT CGG TCC TCC GAT CGT				
GGG GTA CAA CAC GTT TTT TCC CCA ATC GAG GAA GCC ACC ACC CTA GCA				
2500	2510	2520	2530	2540
*	*	*	*	*
TGT CAG AAG TAA GTT GGC CGC AGT GTT ATC ACT CAT CGT TAT GGC AGC				
ACA GTC TTC ATT CAA CGG GCG TCA CAA TAG TGA GTA CCA ATA CGG TCG				
2550	2560	2570	2580	2590
*	*	*	*	*
ACT GCA TAA TTC TCT TAC TGT CAT CGC ATC CGT AAG ATG CTT TTC TGT				
TGA CGT ATT AAG AGA ATG ACA GTA CGG TAG CGA TTC TAC GAA AAG ACA				
2600	2610	2620	2630	2640
*	*	*	*	*
GAC TGG TGA GTA CTC AAC CAA GTC ATT CTC AGA ATA GTG TAT GCG CGC				
CTG ACC ACT CAT GAC TTG GTT CAG TAA GAC TCT TAT CAC ATA CGC CGC				

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FIG. 5 F

2650 2660 2670 2680 2690
 ACC GAG TTG CTC TTG CCC GGC GTC AAC ACG GGA TAA TAC CGC CCC ACA
 TGG CTC AAC GAG AAC GGG CGG CAG TTG TGC CCT ATT ATG GCG CGG TGT

 2700 2710 2720 2730
 TAG CAG AAC TTT AAA AGT GCT CAT CAT TGG AAA ACG TTC TTC GGG GCG
 ATC GTC TTG AAA TTT TCA CGA GTA ACC TTT TGC AAG AAG CCC CGC

 2740 2750 2760 2770 2780
 AAA ACT CTC AAG GAT CTT ACC GCT GTT GAG ATC CAG TTC GAT GTA ACC
 TTT TGA GAG TTC CTA GAA TGG CGA CAA CTC TAG GTC AAG CTA CAT TGG

 2790 2800 2810 2820 2830
 CAC TCG TGC ACC CAA CTG ATC TTC AGC ATC TTT TAC TTT CAC CAG CGT
 GTG AGC ACC TCG GTT GAC TAG AAG TCG TAG AAA ATG AAA GTG GTC GCA

 2840 2850 2860 2870 2880
 TTC TGG GTG AGC AAA AAC AGG AAG GCA AAA TGC CGC AAA AAA GGG AAT
 AAG ACC CAC TCG TTT TTG TCC TTC CGT TTT ACG GCG TTT TTT CCC TTA

 2890 2900 2910 2920 2930
 AAC GGC GAC ACC GAA ATG TTG AAT ACT CAT ACT CTT CCT TTT TCA ATA
 TTC CCG CTG TGC CTT TAC AAC TTA TGA GTA TGA GAA GGA AAA AGT TAT

 2940 2950 2960 2970
 TTA TTG AAG CAT TTA TCA CGG TTA TTG TCT CAT GAG CGG ATA CAT ATT
 AAT AAC TTC GTA AAT AGT CCC AAT AAC AGA GTA CTC CGC TAT GTA TAA

 2980 2990 3000 3010 3020
 TGA ATG TAT TTA GAA AAA TAA ACA AAT AGC CGT TCC CGG CAC ATT TCC
 ACT TAC ATA AAT CTT TTT ATT TGT TTA TCC CCA AGG CGC GTG TAA AGG

 3030 3040 3050 3060 3070
 CCC AAA AGT GCC ACC TGA CGT CTA AGA AAC CAT TAT TAT CAT GAC ATT
 GGC TTT TCA CGG TGG ACT GCA GAT TCT TTG GTA ATA ATA GTA CTG TAA

 3080 3090 3100 3110 3120
 AAC CTA TAA AAA TAG CGG TAT CAC GAG GCC CTG ATG GCT CTT TGC GGC
 TTG GAT ATT TTT ATC CGC ATA GTG CTC CGG GAC TAC CGA GAA ACG CGG

 3130 3140 3150 3160 3170
 ACC CAT CGT TCG TAA TGT TCC GTG GCA CGG AGC ACA ACC CTC AAG AGA
 TGG GTA GCA AGC ATT ACA AGG CAC CGT GGC TCC TGT TGG GAG TTC TCT

 3180 3190 3200 3210
 AAA TGT AAT CAC ACT CGC TCA CCT TCG CGT GGG CCT TTC TGC GTT TAT
 TTT ACA TTA GTG TGA CGG AGT GGA AGC CCA CCC GGA AAG AGC CAA ATA

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FIG. 5 G

3220 3230 3240 3250 3260
 * * * * *
 AAG GAG ACA CTT TAT GTT TAA GAA GGT TCG TAA ATT CCT TGC GGC TTT
 TTC CTC TGT GAA ATA CAA ATT CTT CCA ACC ATT TAA GGA ACG CCG AAA

 3270 3280 3290 3300 3310
 * * * * *
 GGC AGC CAA GCT AGA GAT CCG GCT GTG GAA TGT GTG TCA GTT AGG GTG
 CCG TCG GTT CGA TCT CTA GGC CGA CAC CTT ACA CAC ACT CAA TCC CAC

 3320 3330 3340 3350 3360
 * * * * *
 TGG AAA GTC CCC ACG CTC CCC AGC AGG CAG AAG TAT GCA AAG CTT GCA
 ACC TTT CAG GGG TCC GAG GGG TCG TCC GTC TTC ATA CGT TTC GTC CGT

 3370 3380 3390 3400 3410
 * * * * *
 TCT CAA TTA GTC AGC AAC CAG GCT CCC CAG CAG GCA GAA GTA TGC AAA
 AGA GTT AAT CAC TCC TTG GTC CGA GGG GTC GTC CGT CTT CTT ACG TTT

 3420 3430 3440 3450 3460
 * * * * *
 GCA TGC ATC TCA ATT AGT CAG CAA CCA TAG TCC CCC CCC TAA CTC CGC
 CGT ACG TAG AGT TAA TCA GTC GTT GGT ATC AGG GGG GGG ATT GAG CGC

 3460 3470 3480 3490 3500
 * * * * *
 CCA TCC CGC CCC TAA CTC CGC CCA GTT CGG CCC ATT CTC CGC CCC ATG
 CGT AGG CGC GGG ATT GAG CGG GGT CAA CGC CCC TAA GAG CGG GGG TAC

 3510 3520 3530 3540 3550
 * * * * *
 GCT GAC TAA TTT TTT TTA TTT ATG CAG AGC CGG AGG CGG CCT CGG CCT
 CGA CTG ATT AAA AAA AAT AAA TAC GTC TCC CGC TCC CGC GGA GCC GGA

 3560 3570 3580 3590 3600
 * * * * *
 CTG AGC TAT TCC AGA AGT AGT GAG GAG GCT TTT TTG GAG CGC TAG GCT
 GAC TCG ATA ACG TCT TCA TCA CTC CTC CGA AAA AAC CTC CGG ATC CGA

 3610 3620 3630 3640 3650
 * * * * *
 TTT GCA AAA AGC TAG CTT CGG CGC ACC GCT CAG AGC ACC TTC CAC CAT
 AAA CGT TTT TCG ATC GAA CCC CGG TGG CGA GTC TCG TGG AAG GTG GTA

 3660 3670 3680 3690 3700
 * * * * *
 CGC CAC CTC AGC AAG TTC CCA CTT GAA CAA AAA CAT CAA GCA AAT GTA
 CGG GTG GAG TCG TTC AAG GGT GAA CTT GTT TTT GTA GTT CGT TTA CAT

 3700 3710 3720 3730 3740
 * * * * *
 CTT GTG CCT CGC CCA CGG TGA GAA AGT CCA AGC CAT GTA TAT CTG GGT
 GAA CAC CGA CGG CGT CCC ACT CTT TCA CGT TCG GTA CAT ATA GAC CCA

 3750 3760 3770 3780 3790
 * * * * *
 TGA TCG TAC TCG AGA AGG ACT CGG CTG CAA AAC CGG CAC CCT GGA CTG
 ACT ACC ATC ACC TCT TCC TGA CGC GAC GTT TTG CGC GTG GGA CCT GAC

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FIG. 5 H

3800 3810 3820 3830 3840
 * * * * *
 TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC
 ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG

 3850 3860 3870 3880 3890
 * * * * *
 TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC
 ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG

 3900 3910 3920 3930
 * * * *
 TGT TGC CAT GTT TCG GGA CCC CTT CCG CAG AGA TCC CAA CAA GCT GGT
 ACA ACG GTA CAA ACC CCT GGG GAA GGC GTC TCT AGG GTT GTT CCA CCA

 3940 3950 3960 3970 3980
 * * * * *
 GTT CTG TGA ACT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT
 CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CCG ACC TCT CTC GTT AAA

 3990 4000 4010 4020 4030
 * * * * *
 AAG GCA CTC GTC TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC
 TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG

 4040 4050 4060 4070 4080
 * * * * *
 CTG GTT TCG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA
 GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTC TCT ACC CGT

 4090 4100 4110 4120 4130
 * * * * *
 CCC TTT TCG TTG GGC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA
 GGG AAA ACC AAC CGG AAG GTT ACC GAA AGG ACC CGG GGT TCC AGG CAT

 4140 4150 4160 4170
 * * * *
 TTA CTG TCG TGT GGC CGC AGA CAA ACC CTA TGG CAG GGA TAT CGT GGA
 AAT GAC ACC ACA CCC GCG TCT GTT TCG GAT ACC GTC CCT ATA GCA CCT

 4180 4190 4200 4210 4220
 * * * * *
 CCC TCA CTA CGG CGC CTC CTT GTA TCC TGG GGT CAA GAT TAC AGG AAC
 CGG AGT GAT GGC CGC GAC GAA CAT ACC ACC CCA GTT CTA ATG TCC TTG

 4230 4240 4250 4260 4270
 * * * * *
 AAA TGC TGA GGT CAT GGC TCC CCA GTG GGA ACT CCA AAT AGG ACC CTG
 TTT ACG ACT CCA GTA CGG ACC GGT CAC CCT TGA GGT TTA TCC TGG GAC

 4280 4290 4300 4310 4320
 * * * * *
 TGA AGG AAT CGG CAT GGG AGA TCA TCT CTC GGT GGC CGG TTT CAT CTT
 ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CGG GGC AAA GTA GAA

 4330 4340 4350 4360 4370
 * * * * *
 NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA
 NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT CGG GTT

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FIG. 5 I

4380 4390 4400 4410
 * * * * *
 GCC CAT TCC TGG GAA CTG GAA TGG TGC AGG CTG CCA TAC CAA CTT TAG
 CCG GTA AGG ACC CTT GAC CTT ACC ACG TCC GAC GGT ATG GTT GAA ATC

 4420 4430 4440 4450 4460
 * * * * *
 CAC CAA GGC CAT GCG GGA GGA GAA TGG TCT GAA GCA CAT CGA GGA GGC
 GTG GTT CCG GTA CGC CCT CTT ACC AGA CTT CGT GTA GCT CCT CGC

 4470 4480 4490 4500 4510
 * * * * *
 CAT CGA GAA ACT AAG CAA GCG GCA CGG GTA CCA CAT TCG AGC CTA CGA
 GTA GCT CTT TGA TTC GTT CGC CGT GGC CAT GGT GTA AGC TCG GAT GCT

 4520 4530 4540 4550 4560
 * * * * *
 TCC CAA GGG GGG CCT GGA CAA TGC CGG TGG TCT GAC TGG GTT CCA CGA
 AGG GTT CCC CCC GGA CCT GTT ACG GGC ACC AGA CTG ACC CAA GGT GCT

 4570 4580 4590 4600 4610
 * * * * *
 AAC GTC CAA CAT CAA CGA CTT TTC TGC TGG TGT CGC CAA TCG CAG TGC
 TTG CAG GTT GTA GTT GCT GAA AAG ACG ACC ACA CGG GTT AGC GTC ACC

 4620 4630 4640 4650
 * * * * *
 CAG CAT CGG CAT TCC CGG GAC TGT CGG CCA CGA GAA GAA AGG TTA CTT
 GTC GTA GGC GTA AGG GGC CTG ACA GCC GGT CCT CTT CTT TCC AAT GAA

 4660 4670 4680 4690 4700
 * * * * *
 TGA AGA CGG CGG CGG CTC TGC CAA TTC TGA CCC CTT TGC AGT GAC AGA
 ACT TCT GGC GGC GGG GAG GTT AAC ACT CGG GAA ACG TCA CTG TCT

 4710 4720 4730 4740 4750
 * * * * *
 AGC CAT CGT CGG CAC ATG CCT TCT CAA TGA GAC TGG CCA CGA CGG CTT
 TCG GTA CCA CGC GTG TAC CGA AGA GTT ACT CTG ACC GGT GCT CGG GAA

 4760 4770 4780 4790 4800
 * * * * *
 CCA ATA CAA AAA CTA ATT AGA CTT TGA GTG ATC TTC AGC CTT TCC TAG
 GGT TAT GTT TTT GAT TAA TCT GAA ACT CAC TAG AAC TCC GAA AGG ATC

 4810 4820 4830 4840 4850
 * * * * *
 TTC ATC CCA CGG CGG CGG AGA GAG ATC TTT GTG AAG GAA CCT TAC TTC
 AAG TAG GGT CGG CGG GGG TCT CTC TAG AAA CAC TTC CTT CGA ATG AAG

 4860 4870 4880 4890
 * * * * *
 TGT CGT GTG ACA TAA TTC GAC AAA CTA CCT ACA GAG ATT TAA AGC TCT
 ACA CCA CAC TGT ATT AAC CTC TTT GAT CGA TGT CTC TAA ATT TCC AGA

 4900 4910 4920 4930 4940
 * * * * *
 AAG GTA AAT ATA AAA TTT TTA AGT GTA TAA TGT GTT AAA CTA CTG ATT
 TTC CAT TTA TAT TTT AAA AAT TCA CAT ATT ACA CAA TTT GAT GAC TAA

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FIG. 5 J

4950 4960 4970 4980 4990
 * * * * *
 CTA ATT GTT TGT GTA TTT TAG ATT CCA ACC TAT GGA ACT GAT GAA TGG
 GAT TAA CAA ACA CAT AAA ATC TAA GGT TGG ATA CCT TGA CTA CTT ACC

 5000 5010 5020 5030 5040
 * * * * *
 GAG CAG TGG TGG AAT GCC TTT AAT GAG GAA AAC CTG TTT TGC TCA GAA
 CTC GTC ACC ACC TTA CGG AAA TTA CTC CTT TTG GAC AAA ACG AGT CTT

 5050 5060 5070 5080 5090
 * * * * *
 GAA ATG CCA TCT AGT GAT GAG CCT ACT GCT GAC TCT CAA CAT TCT
 CTT TAC GGT AGA TCA CTA CTC CGA TGA CGA CTG AGA GTT GTA AGA

 5100 5110 5120 5130 5140
 * * * * *
 ACT CCT CCA AAA AAG AAG AGA AAG GAA GAA GAC CCC AAG GAC TTT CCT
 TGA GGA GGT TTT TTC TTC TCT TTC CAT CTT CTG GGG TTC CTG AAA GGA

 5150 5160 5170 5180 5190
 * * * * *
 TCA GAA TTG CTA AGT TTT TTG AGT CAT GCT GTG TTT AGT AAT AGA ACT
 AGT CTT AAC GAT TCA AAA AAC TCA GAA CGA CAC AAA TCA TTA TCT TGA

 5200 5210 5220 5230 5240
 * * * * *
 CTT GCT TGC TTT CCT ATT TAC ACC ACA AAG GAA AAA GCT GCA CTG CTA
 GAA CGA ACC AAA CGA TAA ATG TGG TGT TTC CTT TTT CGA CGT GAC GAT

 5250 5260 5270 5280 5290
 * * * * *
 TAC AAG AAA ATT ATG GAA AAA TAT TCT GAA ACC TTT ATA AGT AGG CAT
 ATG TTC TTT TAA TAC CTT TTT ATA AGA CAT TGG AAA TAT TCA TCC GAA

 5300 5310 5320 5330 5340
 * * * * *
 AAC AGT TAT AAT CAT AAC ATA CTG TTT TTT CTT ACT CCA CAC AGG CAT
 TTG TCA ATA TTA GAA TTG TAT GAC AAA AAA GAA TGA GGT GTC TCC GAA

 5350 5360 5370 5380 5390
 * * * * *
 AGA GTC TCT CCT ATT AAT AAC TAT GCT CAA AAA TTG TGT ACC TTT AGC
 TCT CAC AGA CGA TAA TTA TTG ATA CGA GTC TTT AAC ACA TGG AAA TCG

 5400 5410 5420 5430 5440
 * * * * *
 TTT TTA ATT TGT AAA GGG GTT AAT AAG GAA TAT TTG ATG TAT AGT GCC
 AAA AAT TAA ACA TTT CCC CAA TTA TTC CTT ATA AAC TAC ATA TCA CGG

 5450 5460 5470 5480 5490
 * * * * *
 TTG ACT AGA GAT CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT
 AAC TGA TCT CTA GAA TTA GTC CGT ATG GTC TAA ACA TCT CCA AAA TGA

 5500 5510 5520 5530 5540
 * * * * *
 TGC TTT AAA AAA CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT
 AGC AAA TTT TTT GGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA

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FIG. 5 K

5530 5540 5550 5560 5570
 GAA TGC AAT TGT TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA
 CTT ACG TTA ACA ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT

 5580 5590 5600 5610
 CAA ATA AAG CAA TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC
 GTT TAT TTC GTT ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG

 5620 5630 5640 5650 5660
 ACT GCA TTC TAG TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA
 TGA CGT AAG ATC AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT

 5670 5680 5690 5700 5710
 TGT CTG GAT CTC TAG CTT CGT GTC AAG GAC CGT GAC TCC AGT GAA TAA
 ACA GAC CTA GAG ATC GAA GCA CAG TTC CTG CCA CTG ACC TCA CTT ATT

 5720 5730 5740 5750 5760
 TAA AAT GTG TGT TTG TCC GAA ATA CGC GTT TTG AGA TTT CTG TCG CCG
 ATT TTA CAC ACA AAC AGG CTT TAT CCC CAA AAC TCT AAA GAC AGC GGC

 5770 5780 5790 5800 5810
 ACT AAA TTC ATG TCG CGC GAT AGT GGT GTT TAT CCC CGA TAG AGA TGG
 TGA TTT AAG TAC ACC GCG CTA TCA CCA CAA ATA CGC GCT ATC TCT ACC

 5820 5830 5840 5850
 CGA TAT TCG AAA AAT CGA TAT TTG AAA ATA TGG CAT ATT GAA AAT GTC
 GCT ATA ACC TTT TTA GCT ATA AAC TTT TAT ACC GTA TAA CTT TTA CAG

 5860 5870 5880 5890 5900
 GCC GAT GTG AGT TTC TGT GTA ACT GAT ATC CCC ATT TTT CCA AAA GTG
 CGG CTA CAC TCA AAG ACA CAT TGA CTA TAG CGG TAA AAA CGT TTT CAC

 5910 5920 5930 5940 5950
 ATT TTT CGG CAT ACC CGA TAT CTG CGC ATA CGG CTT ATA TCG TTT ACC
 TAA AAA CCC GTA TCC GCT ATA GAC CGC TAT CGC GAA TAT AGC AAA TGC

 5960 5970 5980 5990 6000
 CGG GAT CGC GAT AGA CGA CTT TGG TGA CTT CGG CGA TTC TGT GTG TCG
 CCC CTA CGG CTA TCT GCT GAA ACC ACT GAA CCC GCT AAG ACA CAC AGC

 6010 6020 6030 6040 6050
 CAA ATA TCC CAG TTT CGA TAT AGG TGA CAG ACC ATA TGA CGC TAT ATC
 GTT TAT AGC GTC AAA GCT ATA TCC ACT GTC TGC TAT ACT CGG ATA TAG

 6060 6070 6080 6090
 CGC GAT AGA CGC GAC ATC AAG CTC CGA CAT CGC CGA TCC ATA TCG ATC
 CGG CTA TCT CGG CTC TAG TTC GAC CGT GTA CGG GTT ACC TAT AGC TAG

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FIG. 5 L

6100	6110	6120	6130	6140
* * * * *				
TAT ACA TTG AAT CAA TAT TGG CCA TTA GCC ATA TTA TTC ATT GGT TAT				
ATA TGT AAC TTA GTT ATA ACC GGT AAT CGG TAT AAT AAG TAA CCA ATA				
6150	6160	6170	6180	6190
* * * * *				
ATA GCA TAA ATC AAT ATT GGC TAT TGG CCA TTG CAT ACG TTG TAT CCA				
TAT CGT ATT TAG TTA TAA CCG ATA ACC GGT AAC GTA TGC AAC ATA GGT				
6200	6210	6220	6230	6240
* * * * *				
TAT CAT AAT ATG TAC ATT TAT ATT GGC TCA TGT CCA ACA TTA CCC CCA				
ATA GTA TTA TAC ATG TAA ATA TAA CCG AGT ACA GGT TGT AAT GGC GGT				
6250	6260	6270	6280	6290
* * * * *				
TGT TGA CAT TGA TTA TTG ACT AGT TAT TAA TAG TAA TCA ATT ACG GGG				
ACA ACT GTA ACT AAT AAC TGA TCA ATA ATT ATC ATT AGT TAA TGC CCC				
6300	6310	6320	6330	
* * * * *				
TCA TTA GTT CAT AGC CCA TAT ATG GAG TTC CGC GTT ACA TAA CTT ACG				
AGT AAT CAA GTA TCG GGT ATA TAC CTC AAG CGC CAA TGT ATT GAA TGC				
6340	6350	6360	6370	6380
* * * * *				
GTA AAT GGC CGG CCT GGC TGA CGG CCC AAC GAC CCC CGC CCA TTG ACG				
CAT TTA CGG GCA CGG ACT GGC GGG TTG CTG CGG CGT AAC TGC				
6390	6400	6410	6420	6430
* * * * *				
TCA ATA ATG ACG TAT GTT CCC ATA GTA ACG CCA ATA CGG ACT TTC CAT				
AGT TAT TAC TGC ATA CAA CGG TAT CAT TGC GGT TAT CCC TGA AAG GTA				
6440	6450	6460	6470	6480
* * * * *				
TGA CGT CAA TGG GTG GAG TAT TTA CGG TAA ACT GCC CAC TTG GCA GTA				
ACT GCA GTT ACC CAC CTC ATA AAT GCC ATT TGA CGG GTG AAC CGT CAT				
6490	6500	6510	6520	6530
* * * * *				
CAT CAA GTG TAT CAT ATG CCA AGT ACG CCC CCT ATT GAC GTC AAT GAC				
GTA GTT CAC ATA GTA TAC GGT TCA TGC CGG CGA TAA CTG CAG TTA CTG				
6540	6550	6560	6570	
* * * * *				
GGT AAA TGG CCC CGC TGG CAT TAT GCC CAG TAC ATG ACC TTA TGG GAC				
CCA TTT ACC CGG CGG ACC GTA ATA CGG GTC ATG TAC TGG AAT ACC CTG				
6580	6590	6600	6610	6620
* * * * *				
TTT CCT ACT TGG CAG TAC ATC TAC GTA TTA GTC ATC GCT ATT ACC ATG				
AAA CGA TGA ACC GTC ATG TAG ATG CAT AAT CAG TAG CGA TAA TGG TAC				
6630	6640	6650	6660	6670
* * * * *				
GTC ATG CGG TTT TGG CAG TAC ATC AAT GGG CGT CGA TAG CGG TTT GAC				
CAC TAC CGC AAA ACC GTC ATG TAG TTA CCC CGA CCT ATC CGC AAA CTC				

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FIG. 5 M

6680 6690 6700 6710 6720
 TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG
 AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC

 6730 6740 6750 6760 6770
 TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC
 AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG

 6780 6790 6800 6810
 GCC CCA TTG ACG CAA ATG CGC GGT AGG CGT GTA CGG TGG GAG GTC TAT
 CGG GGT AAC TCC GTT TAC CGG CCA TCC GCA CAT CCC ACC CTC CAG ATA

 6820 6830 6840 6850 6860
 ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GGC TGG AGA CCC CAT
 TAT TCG TCT CCA GCA AAT CAC TTG GCA GTC TAG CGG ACC TCT CCC GTA

 6870 6880 6890 6900 6910
 CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC
 GGT CGC ACA AAA CTG GAG GTA TCT TCT GTC CCC CTG GCT AGG TCG GAG

 6920 6930 6940 6950 6960
 CGC CGC CGG GAA CGG TGC ATT CGA ACC CGG ATT CCC CGT GGC AAC AGT
 CGC CGC CGC CTT GGC ACC TAA CCT TGC GGC TAA CGG CGA CGG TTC TCA

 6970 6980 6990 7000 7010
 GAC GTA AGT ACC CGC TAT AGA GTC TAT AGG CCC ACC CGC TTG GCT TCT
 CTC CAT TCA TGG CGG ATA TCT CAG ATA TCC CGG CGG AAC CGA AGA

 7020 7030 7040 7050
 TAT GCA TCC TAT ACT GTT TTT CGC TTG CGG TCT ATA CAC CCC CGC TTG
 ATA CGT ACC ATA TGA CAA AAA CGG AAC CCC AGA TAT GTG CGG CGC AAC

 7060 7070 7080 7090 7100
 CTC ATC TTA TAG GTG ATC GTA TAG CTT ACC CTA TAG GTG TGG TTG ATT
 GAG TAC AAT ATC CAC TAC CAT ATC GAA TCC GAT ATC CAC ACC CAA TAA

 7110 7120 7130 7140 7150
 GAC CAT TAT TGA CGA CTC CCC TAT TGG TGA CGA TAC TTT CGA TTA CTA
 CTC CTA ATA ACT GGT GAC CGC ATA ACC ACT GCT ATG AAA CGT AAT GAT

 7160 7170 7180 7190 7200
 ATC CAT AAC ATG GCT CTT TCC CAC AAC TCT CTT TAT TGG CTA TAT CGC
 TAG GTA TTG TAC CGA GAA ACC CTC TTG AGA GAA ATA ACC GAT ATA CGG

 7210 7220 7230 7240 7250
 ATT ACA CTG TCC TTG AGA GAC TGA CAC CGA CTC TGT ATT TTT ACA CGA
 TTA TGT GAC AGG AAG TCT CTC ACT GTG CCT GAG ACA TAA AAA TGT CCT

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FIG. 5 N

7260	7270	7280	7290	
TGG GGT CTC ATT TAT TAT TTA CAA ATT CAC ATA TAC AAC ACC ACC GTC ACC CCA GAG TAA ATA ATA AAT GTT TAA GTG TAT ATG TTG TGG TGG CAG				
7300	7310	7320	7330	7340
CCC AGT GCC CCC ACT TTT TAT TAA ACA TAA CGT GGG ATC TCC ACG CGA GGG TCA CGG GCG TCA AAA ATA ATT TGT ATT GCA CCC TAG AGG TGC GCT				
7350	7360	7370	7380	7390
ATC TCG GGT ACG TGT TCC GGA CAT CGG CTC TTC TCC GGT AGC GGC GGA TAG AGC CCA TGC ACA AGG CCT GTA CCC GAG AAC AGG CCA TCG CGG CCT				
7400	7410	7420	7430	7440
GCT TCT ACA TCC GAG CCC TGC TCC CAT GCC TCC ACC GAC TCA TGG TCG CGA AGA TGT AGG CTC GGG AGC AGG GTA CGG AGG TCG CTG AGT ACC AGC				
7450	7460	7470	7480	7490
CTC GGC AGC TCC TTG CTC CTA ACA GTG GAG GGC AGA CTT AGG CAC AGC GAG CGG TCG AGG AAC GAG GAT TGT CAC CTC CGG TCT GAA TCC GTC TCG				
7500	7510	7520	7530	
ACG ATG CCC ACC ACC ACC ACT GTG CGG CAC AAG GGC GTG GCG GTC GAA TCC TAC GGG TGG TCG TGG TCA CAC CCC GTG TTC CGG CAC CGC CAT CCC				
7540	7550	7560	7570	7580
TAT GTG TCT GAA AAT GAG CTC GGG GAG CGG CCT TGC ACC GCT GAC GCA ATA CAC AGA CTT TTA CTC GAG CCC CTC GCC CGA ACC TGG CGA CTG CGT				
7590	7600	7610	7620	7630
TTT CGA AGA CTT AAG GCA CGG GCA GAA GAT GCA CCC AGC TGA GTT AAA CCT TCT GAA TTC CGT CGG CGT CTT CTT CTA CGT CGG TCG ACT CAA				
7640	7650	7660	7670	7680
CTT GTG TTC TGA TAA GAG TCA GAG GTC ACT CCC GTT GCG GTG CTG TTA CAA CAC AAG ACT ATT CTC ACT CTC CAT TGA CGG CAA CGC CAC GAC AAT				
7690	7700	7710	7720	7730
ACG GTG GAG CGC ACT GTC GTC TGA GCA GTC CTC GTT CCT GCT CCC CGG CGC TGC CAC CTC CGG TCA CAT CAG ACT CGT CAT GAG CAA CGA CGG CGC CGG				
7740	7750	7760	7770	
GCC ACC AGA CAT AAT AGC TGA CAG ACT AAC AGA CTC TTC CTT TCC ATC CGG TGG TCT GTC TTA TCG ACT GTC TGA TTG TCT GAC AAC GAA AGG TAC				
7780	7790	7800	7810	7820
GGT CTT TTC TGC ACT CAC CGT CCT TGA CAC GAA CCT TGG CCT GCA CGT CCA GAA AAG ACC TCA GTG GCA ACT GTG CTT CGA ACC CGA CGT CGA				

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FIG. 5 O

7830

7840

7850

7860

CGA TCG ACT CTA GAG GAT CGA TCC CGG GGC GAG CTC G
CCT AGC TGA GAT CTC CTA CCT AGG GGC CGG CTC GAG C

FIG. 6
anti-TF BINDING ASSAY

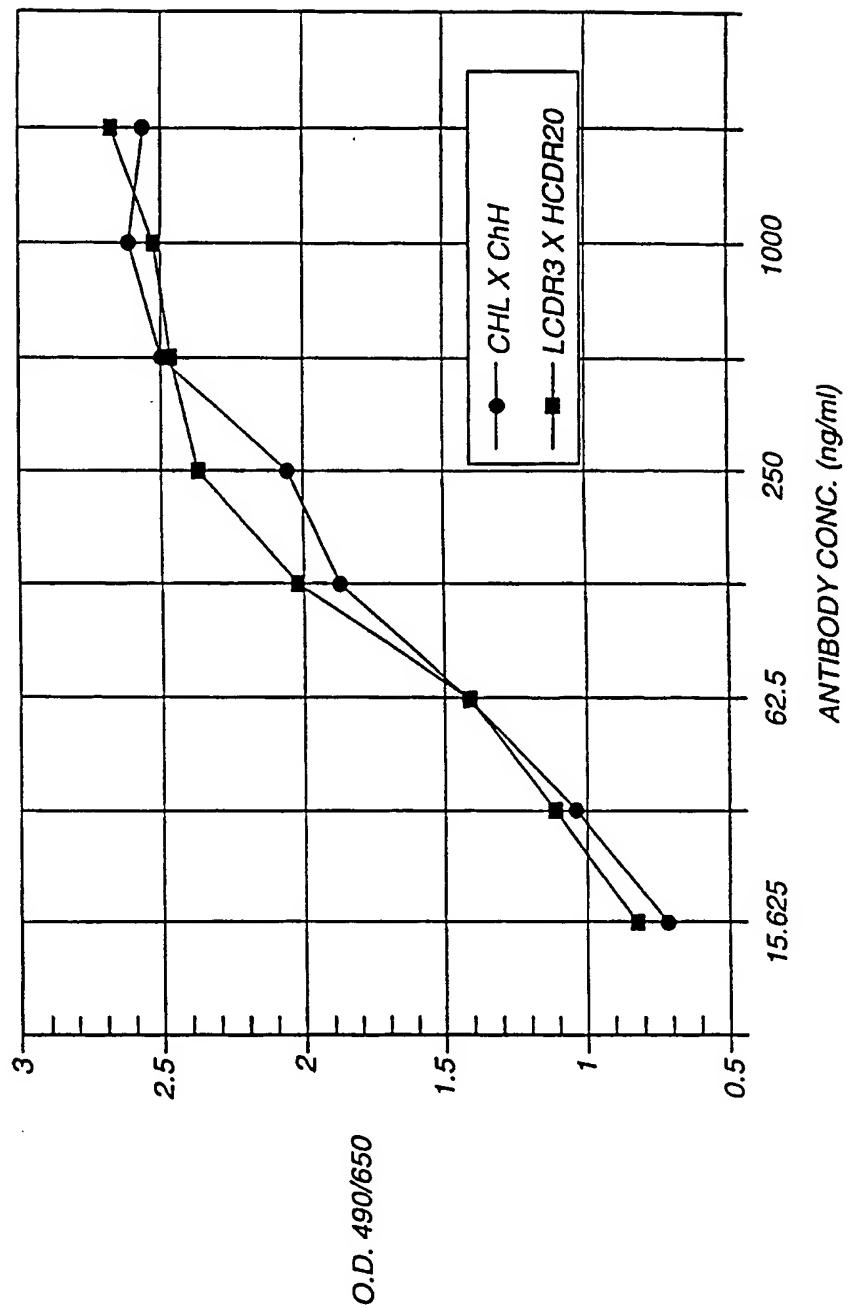
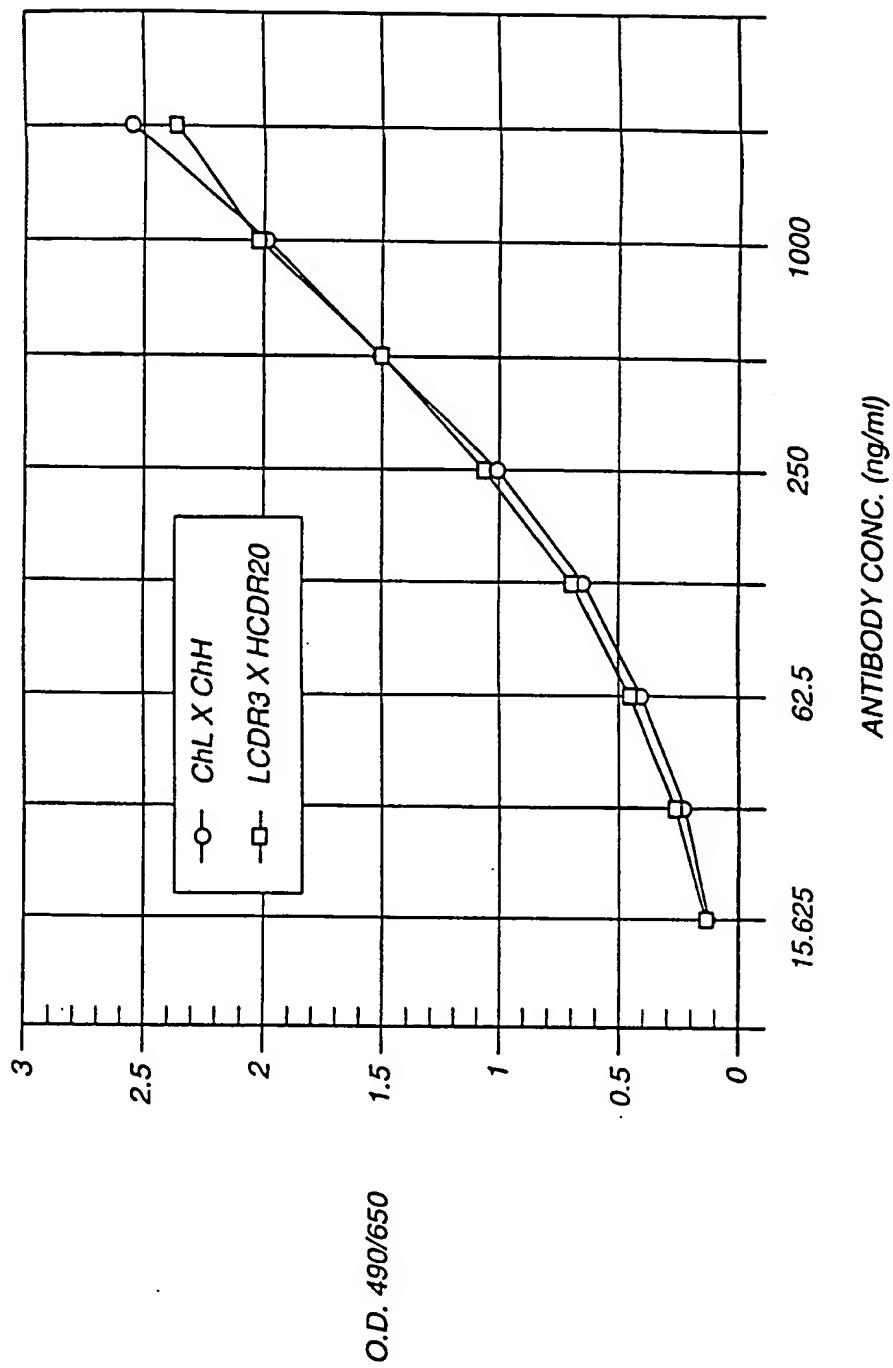
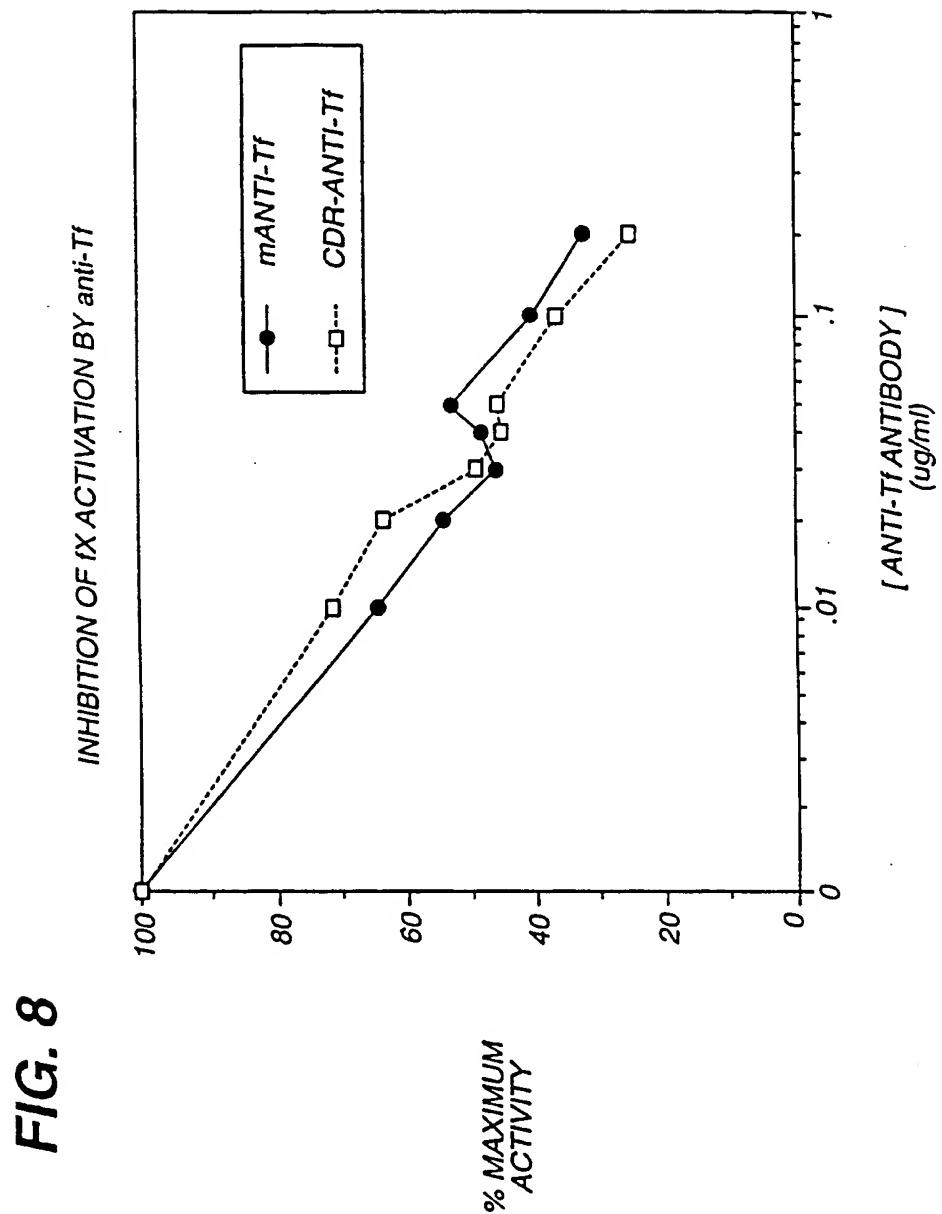
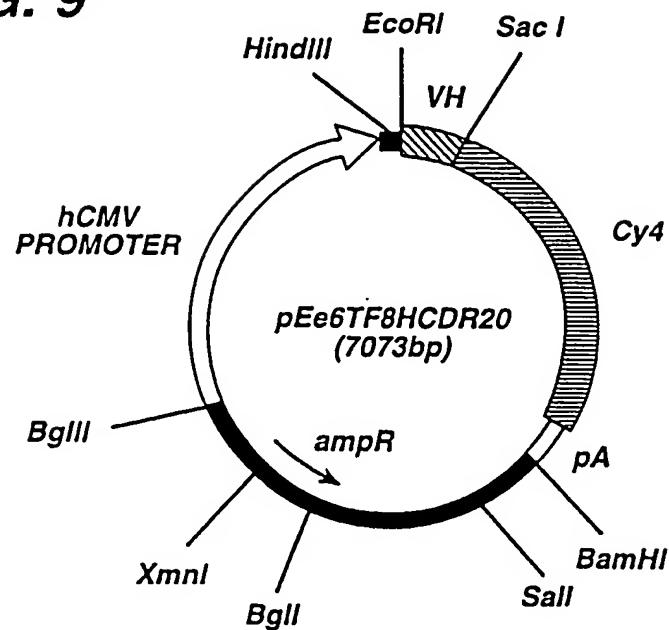
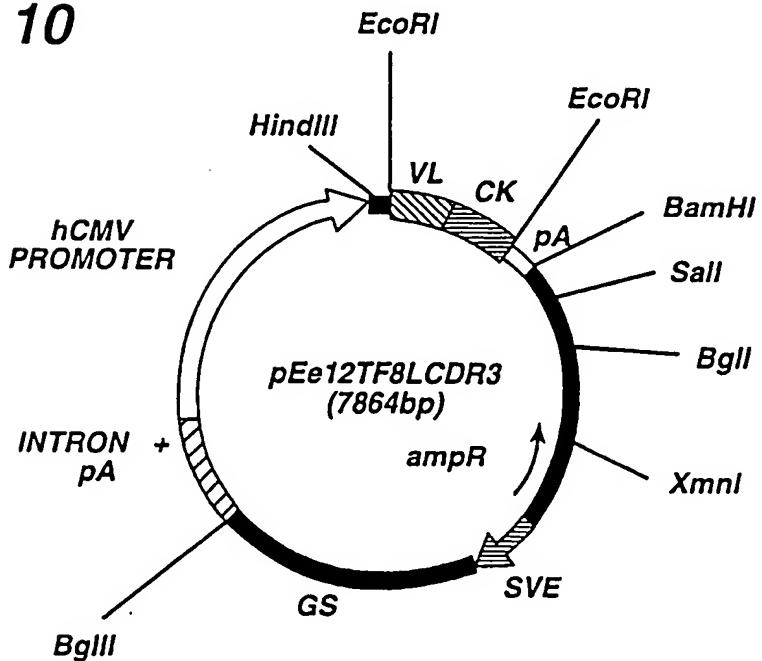


FIG. 7
anti-TF COMPETITION ASSAY



41/41

FIG. 9**FIG. 10**

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/09287

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/13 C07K16/36 C07K16/46 A61K39/395 //C12N5/10,
C12N15/85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 91 09968 A (CELLTECH LIMITED) 11 July 1991 see examples see claims ---	1-37
Y	WO 88 07543 A (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 6 October 1988 see claims ---	1-37
A	WO 94 11029 A (THE SCRIPPS RESEARCH INSTITUTE ET AL.) 26 May 1994 see claims ---	1-37
A	WO 94 05328 A (THE SCRIPPS RESEARCH INSTITUTE) 17 March 1994 see examples see claims ---	1-37
		-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

1 Date of the actual completion of the international search

Date of mailing of the international search report

15 October 1996

08.11.96

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Fax (+ 31-70) 340-3016

Authorized officer

Nooij, F

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/09287

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF CRYSTAL GROWTH, vol. 122, no. 1-4, August 1992, AMSTERDAM, NL, pages 253-264, XP002015918 W. RUF ET AL.: "Purification, sequence and crystallization of an anti-tissue factor Fab and its use for the crystallization of tissue factor." see abstract see table 1 -----	1-37

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/09287

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 31-35
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 31-35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No	
PCT/US 96/09287	

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9109968	11-07-91	AT-T- AT-T- AU-B- AU-A- AU-B- AU-A- AU-B- AU-A- AU-B- BG-B- CA-A- CA-A- CA-A- DE-D- DE-T- DE-D- DE-T- EP-A- EP-A- EP-A- EP-A- EP-A- ES-T- ES-T- WO-A- WO-A- GB-A,B GB-A,B GB-A,B GB-A,B JP-T- JP-T- JP-T-	129017 124459 664801 6461294 646009 6974091 649645 7033091 631481 7048691 60462 2037607 2046904 2050479 69020544 69020544 69022982 69022982 0460167 0460171 0460178 0620276 0626390 2079638 2074701 9109966 9109967 2246781 2246570 2268744 2268745 4505398 4506458 5500312	15-10-95 15-07-95 30-11-95 22-12-94 03-02-94 24-07-91 02-06-94 24-07-91 26-11-92 24-07-91 28-04-95 07-09-92 22-06-91 22-06-91 03-08-95 18-01-96 16-11-95 28-03-96 11-12-91 11-12-91 11-12-91 19-10-94 30-11-94 16-01-96 16-09-95 11-07-91 11-07-91 12-02-92 05-02-92 19-01-94 19-01-94 24-09-92 12-11-92 28-01-93
WO-A-8807543	06-10-88	US-A- US-A- AU-B- AU-A- EP-A-	5110730 5223427 605864 1627488 0309548	05-05-92 29-06-93 24-01-91 02-11-88 05-04-89

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/09287

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-8807543		FI-A- 954347 GR-A- 88100198 JP-T- 1503438 US-A- 5437864	15-09-95 31-01-89 22-11-89 01-08-95
WO-A-9411029	26-05-94	US-A- 5437864 AU-A- 5671594	01-08-95 08-06-94
WO-A-9405328	17-03-94	AU-A- 5093593	29-03-94